ALLELOPHATIC INFLUENCE OF CERTAIN WEED SPECIES ON SEED OF MAIZE AND SOYBEAN CROPS

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Abstract: *Natural ecological phenomenon of relationship between organisms that can be used for weed control in all annual and perennial crops is called allelophaty. In the future, as a natural phenomenon it can represent one of the mechanisms in biological weed control measures. During 2011 and 2012, in laboratory conditions allelophatic effect of cold aqueous extracts made of underground and above ground parts of Amaranthus retroflexus L. and Abuthilon theophrasti Med. was studied on germination capability of seeds and beginning growth phases of maize and soybean seed. Laboratory tests were set up in randomized block design in four replications. Tests were set up in four concentration ranges of 25, 50, 75 and 100 g/l of aqueous extractions from underground part, i.e. root and aboveground parts, i.e. stem and leaf of weed species A. retroflexus L. and A. theophrasti Med. In control variant maize and soybean seeds were sprayed by distilled water. After germination of crop seeds and subsequent treatment by cold aqueous extract made of these weed species, in next five days followed measurements of epicotyls and hypocotyls length of maize and soybean seeds on a daily basis. Before setting up of the test, studies of germination capability of seeds of the chosen maize and soybean crops were performed in climatic chamber under controlled conditions. Inhibiting effect of the applied aqueous extract from underground part of A. theophrasti Med. proved statistically insignificant on soybean hypocotyls growth in all four studied concentrations. Studied cold extract from above ground A. theophrasti Med. showed high inhibiting effect on soybean epicotyls length in all four tested concentrations. Application of cold extract made from underground part of A. retroflexus L. resulted in high allelophatic effect on germination capability of maize seed, as well as in reduction of maize seed hypocotyls length. On the other hand, treatment of maize seed by cold extract from above ground parts resulted in statistically significant effect in the highest concentration, whereas in the remaining three concentrations significant effect to the growth of maize seed epicotyls was not established.*

Key words: *allelophaty, Amaranthus retroflexus L., Abuthilon theophrasti Med., maize, soybean*

INTRODUCTION

Harmful chemical substances that plants release to the environment reducing their growth and establishment of other plants in their surrounding are called allelopathy. Chemical substances with allelophatic properties have also other ecological features that have impact to degradation and soil fertility, that can be increased or reduced, or their mutual functions can be altered. (INDERJIT *et al*., 2011). Weeds have harmful effect to the grown crops due to release of phytotoxines out of weed seed and decomposition of weed remaining (NARWAL, 2004). Chemical substances with allelophatic effect and their derivates, and even compounds synthesized upon their chemical structure can be used for weed control in ecologically sound manner (SINGH *et al*., 2001). In last

ten year, allelophatic effect of aqueous extracts made of different plant parts and their influence to the other plant species has been intensively studied, with the aim of research of biofertilizers and bioherbicides (QIAN *et al*., 2010). Fruit, leaf, stem and root have allelophatic potential (TINNIN and MULLER, 2006). The main parts for production of allelophatic substances are leaves and it was proved that allelophatic substances in aqueous extracts obtained from leaves show the highest effect, while allelophatic substances in aqueous extracts made of roots show the lowest effect (SISODIA and SIDDIQUI, 2010). Allelophatic effect that affects germination and growth of seedlings differs from weed to weed (HAMAYUN *et al*., 2005). Interaction between weeds and crops is achieved by direct or indirect action of one plant to another. Inhibitory action to the seed germination and development of crops is achieved by synthesis of different allelochemicals (ALEKSIEVA and SERAFIMOV, 2008). In field crops allelopathy can be used after crop rotation, mulching of plant parts and making of extracts for biological control of pests. The application of plant extracts can be used for weed and pests control (YARNIA *et al*., 2009). Lower herbicide rates can reduce development of herbicide resistance of different weed species, while the phenomenon of allelopathy can provide ecological replacement for pesticides in agricultural production (VERMA and RAO, 2006).

MATERIAL AND METHODS

In the period 2011-2012, at localities Bački Maglić and Kać plant material of weed species *Abutilon theophrasti* Med. and *Amaranthus retroflexus* L. was collected. Collected plant material was separated to the underground part (root) and the aboveground parts (stem and leaf). The underground and aboveground parts were separately macerated and extracts were made from both of weed parts, i.e. the underground and the aboveground ones. Extract from the underground and the aboveground parts of *A. retroflexus* L. and *A. theophrasti* Med. were made in a range of concentration ranges of 25.0; 50.0; 75.0 and 100.0 g (biomass/l distilled water). Previously germinated seed of maize and soybean laid on filter paper in Petri dishes were soaked with 8 ml of each extract separately. Petri dishes contained 25 seeds of soybean or maize. Tests were set up with extract concentrations made of both of weed species in four replications, i.e. *A. retroflexus* L. + maize and *A. theophrasti* Med. + soybean. Control variant was sprayed with distilled water. Tests were set up in randomized block design in four replications and each Petri dish contained 25 soybean or maize seeds. During the tests, measurements were performed on daily basis, five days after soaking of the studied crop seed. Studies of allelophatic effect of the studied aqueous extract made of weeds to the given crops were performed by measurements of hypocotyls and epicotyls lengths of the crop seed (MARINOV-SERAFIMOVA *et al*., 2007). Seed surface was sterilized according to the method of Elemar and Filhou, 2005.

RESULTS AND DISCUSSION

During studies of allelophatic effect of aqueous extracts made of underground parts of weed species *A. theophrasti* Med. A pronounced inhibiting effect to soybean hypocotyls growth was established, while application of the extract made of underground parts of the weed did not show statistically significant effect to the epicotyls growth in neither of the studied variants.

In all of the applied extract concentrations made of the aboveground parts of *A. theophrasti* Med., statistically significant difference to hypocotyls growth was proved, while for the epicotyls statistically significant difference was not established.

The average values of hypocotyls length for concentration of 25 g/l were 17.22 mm, which was less in relation to the average determine control value of 20.773 mm. The measured average hypocotyls length after application of extract concentration of 50 g/l was 17.42 mm, while control value was 20.57 mm. Application of higher extract concentrations resulted in lower average values of hypocotyls length, i.e. for concentration of 75 g/l the average hypocotyls length was 16.41 mm, and control value was 20.88 mm. The lowest average hypocotyls length of 16.15 mm in comparison to control variant with value of 21.27 mm was measured in the highest extract concentration of 100 g/l. For extract concentration of 25 g/l of the aboveground parts of *A. theophrasti* L., the average soybean seed hypocotyls length was 20.05 mm, and established average control value was 20.773 mm. In extract concentration range of 50 g/l soybean seed hypocotyls length was 19.82 mm, and control value was 20.57 mm. The applied concentration of extract in a range of 75 g/l resulted in measured average hypocotyls lengths of 20.41 mm, while control value was 20.88 mm. Application of the highest extract concentration resulted in hypocotyls length of 20.74 mm, and control value was 21.18 mm (Figure 1). In the period 2006-2007, at Institute of Forage Crops, Plaven were carried out laboratory tests on allelophatic effect of cold aqueous extracts from certain weed species. Test results with aqueous extracts from fresh and dry biomass of *Amaranthus retroflexus* L., *Chenopodium album* L., *Erigeron canadensis* L. and *Solanum nigrum* L. showed that extracts made of fresh biomass have inhibiting effect of 28.8-81.8% to germination capability and beginning growth stages of soybean, bean and clover (MARINOV-SERAFIMOV(2012). KALINOVA et al (2012) established that differences in the studied parameters increase with the increase of extract concentration. Effect of *Sorghum halepense* (L) Pers. to seed germination capability of soybean and bean seeds was tested, and the obtained results confirmed inhibitory effect of extracts in a range of 28.8-86.3%, depending upon extract concentration.

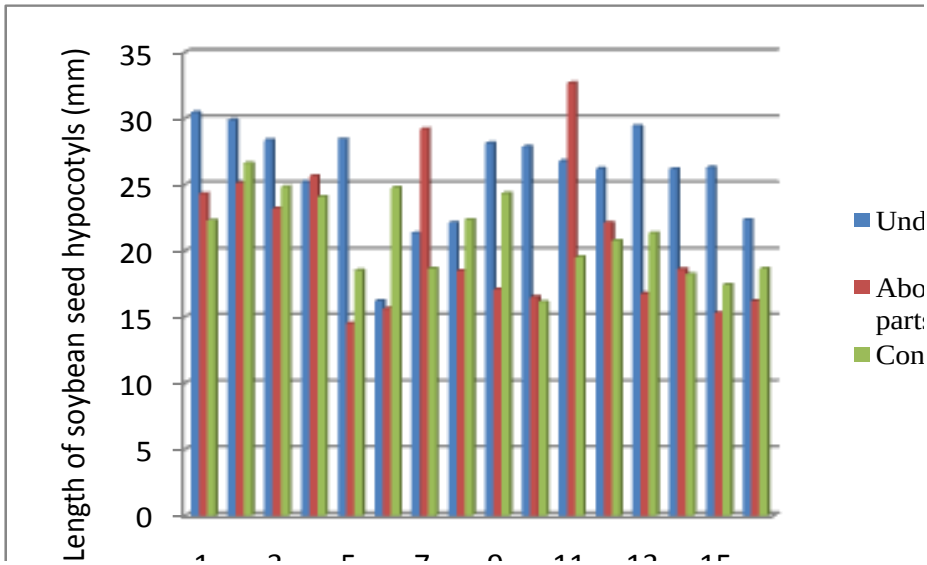

Figure 1: Effect of *Abutilon theophrasti* Med. extract to the length of soybean seed hypocotyls lenght in concentraion of 100 g plant mass/distilled water

Data processing of the results obtained upon applied extract made of the above ground parts of *A. theophrasti* Med. to soybean seed revealed that there were no allelophatic effects to epicotyls growth in all applied combinations. For the applied extract concentration of 25 g/l made of above ground parts of *A. theophrasti* Med. the average established values for epicotyls length was 7.12, and control value was 7.472 mm. The average measured epicotyls length for extract concentration of 50 g/l was 8.423 mm, while the measured control had the value of 7.93 mm. The applied extract concentration of 75 g/l resulted in epicotyls lengths of 7.823 mm, and the average value in control was 7.342 mm. For extract concentration of 100 g/l established value of epicotyls lengths was 7.65 mm, while the control was 7.85 mm. The results of the tested extract made of underground parts of *A. theophrasti* Med. did not show statistically significant effect to the epicotyls lengths. For extract concentration of 25 g/l the measured epicotyls length was 7.448 mm, and established control value was 8.212 mm. For the following concentration of 50 g/l the average epicotyls length was 7.321 mm, and measured average value in control was 7.43 mm. The value of epicotyls length for concentration of 75 g/l was 7.21 mm, with control value of 7.8 mm. For the highest applied concentration of 100 g/l the average epicotyls length was 7.11 mm, while the control was 7.68 mm.

During the studies of allelophatic effect of weed species *Amaranthus retroflexus* L. to the beginning growth and development of maize seed hypocotyls, extract made of the above ground part of the weed inhibited growth of maize seed hypocotyls. The average hypocotyls length for extract concentration of 25 g/l, made of the above ground parts of *A.retroflexus* was 27.5 mm, which was less in comparison to the average control values of 28.96 mm; for extract concentration of 50 g/l established average hypocotyls length was 26.86 mm, and control was 29.18 mm. The

application of higher extract concentration of 75 g/l resulted in average hypocotyls length of 26.53 mm, and control was 28.8 mm. In the highest applied extract concentration of 100 g/l the average maize seed hypocotyls length was the shortest, i.e. 24.7 mm in comparison to control variant of 28.9 mm. In laboratory tests with extract of the underground part of *A. retroflexus* L. values of maize seed hypocotyls length did not significantly differ from control. In laboratory tests, extract made of underground part of *A. retroflexus* L. values of maize seed hypocotyls did not significantly differ from control. For variant of extract concentration of 25 g/l made of underground part of *A.retroflexus* the average measured hypocotyls length was 27.81 mm, while the average control value was 28.96 mm. The established average value of hypocotyls length for extract concentration of 50 g/l was 27.89 mm, and control value was 29.27 mm. After the applied extract in concentration of 75 g/l, the average measured hypocotyls length was 27.18 mm, in comparison to control value of 28.8 mm. The highest applied concentration of 100 g/l resulted in average hypocotyls length of 26.75 mm, while the average length in control was 28.9 mm.

The average value of epicotyls length of 7.23 mm was measured after application of extract made of the above ground parts of *A.retroflexus* L. in concentration of 25 g/l, in comparison to control value of 8.29 mm. The application of extract concentration of 50 g/l resulted in average epicotyls length of 6.81 mm, while the average measured control was 8.09 mm; the applied concentration of 75 g/l had value of 6.98 mm, with average control of 7.81 mm. Application of extract concentration of 100 g/l resulted in average epicotyls length of 5.83 mm, in comparison to control value of 8.16 mm. Effect of extract made of underground part of *A.retroflexus* L. also did not have statistically significant effect to the epicotyls length in extract concentrations of 50 and 75 g/l, while application of extract concentrations of 25 and 100 g/l showed allelophatic effect. After applied extract concentration of 25 g/l the average measured epicotyls length of maize seeds was 6.42 mm, and control value was 8.29 mm. The applied extract concentration of 50 g/l resulted in average maize seed epicotyls of 6.27 mm, and the average control value was 8.09 mm. In the highest applied extract concentration of 100 g/l the average epicotyls length was 5.48 mm, in comparison to control value of 7.68 mm (Figure 2).

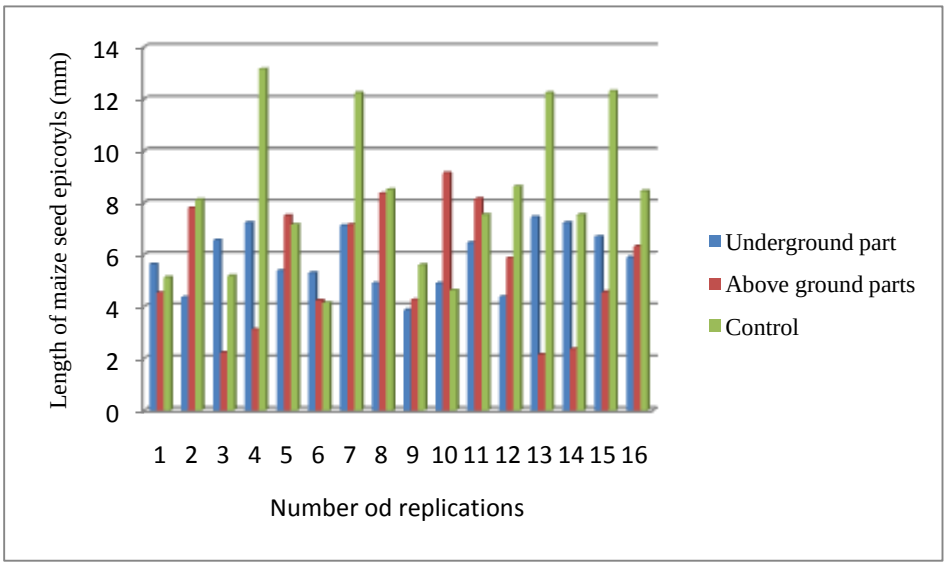

Figure 2: Effect of *Amaranthus retroflexus* L. extract to the length of maize seed epicotyls lenght in concentraion of 100 g plant mass/distilled water

Statistical data processing by program Statistika 10, significant deviations from the average hypocotyls length were determined after treatment with extracts made of underground and above ground parts of *A. theophrasti* Med. The study was performed by t-test method in all four cases in relation to the height of extract concentration. Data processing of the zero hypotheses showed that significant deviation between tested and control group does not exist, while alternative hypothesis provided significantly different results. The extract made of the above ground parts of A. *theophrasti* L. in concentration of 25 g/l resulted in difference in soybean seed hypocotyls of 3.5524 mm, while the value of t-test was 4.7173, with a estimated probability of the error of the first range of 0.00027, which is less than the level of statistical significance of the alpha test of 0.05. On the level of statistically significance of 0.01 it was established that zero-hypothesis is not acceptable, because test results suggest that statistically significant difference does not exist in expected values for extracts made of the underground part of *A. theophrasti* Med. By T-test it was determined that there was no statistically significant difference. The evaluated errors of the first grade were lower from the threshold of 0.05, for the expected values of the studied parameters on the level of statistical difference of 95%. Results of application of extract concentration made of underground part of *A.theophrasti* L. by which soybean seed was treated showed that on the level of statistical difference in t-test, there were no statistically significant differences between tested variants and control. For soybean seed treated by extract in concentration of 25 g/l difference between evaluated and expected values of 0.72625 was evidenced, while the evaluated error of the first grade was 0.345394. From these results, it was determined that there were not enough arguments for rejection of the zero hypotheses.

Processing of data obtained after application of the extract made of the above ground parts of *A.retroflexus* L. In concentration of 25 g/l, revealed variation in maize seed hypocotyls of 2.28950 mm, while the value of t-test was -2.5627, the evaluated possibility of the first grade error was 0.021642, which is less than the level of the alpha test significance=0,05. It was established that on the level of statistical significance of 0.01 there exist enough arguments for not accepting the zero hypothesis. It was also found that on the maize seed hypocotyls growth significant effect have extracts made of the above ground parts of *A. retroflexus* L. In concentrations of 50, 75 and 100 g/l of weed plant material. The evaluated possibility of the error of the first grade for the extract made of the above ground parts of *A. retroflexus* l. In concentrations of 50 g/l was 0.000220, for 75 g/l it was 0.017323, and for concentration of 100 g/l it was 0.001797. On the level of statistical significance 0.01 zero hypotheses for this data is not acceptable.

Maize seed hypocotyls growth was not recorded after application of all extract concentrations made of the underground part of *A.retroflexus* L., and zero hypothesis was not accepted for the evaluated error of the first grade was lower than 0.05. In variant of extract concentration of 25 g/l, deviation in maize seed hypocotyls length was 3.60818 mm, and evaluated possibility of the first grade error was 0.239214, which is higher than the level of statistical significance test alpha = 0.05. Data processing revealed that effect of extract concentrations made of the underground part of *A.retroflexus* L., were not statistically significant on the significance level of 95%.

Results of the t-test of extract in concentration of 100 g/l suggest that there was statistically significant difference between maize seed epicotyls treated by extract of the above ground parts of *A.retroflexus* L. The evaluated possibility of the first grade error was 0.00188, which was less than the level of the statistical significance of the test alpha=0.05. For lower extract concentrations of 25, 50 and 75 g/l made of the above ground parts of *A.retroflexus* L. it was established that there were not enough arguments for rejection of the zero-hypothesis. It was also determined that for these concentrations differences in maize seed epicotyls in comparison to control were not statistically significant if the level of significance was 95%. In experimental variant with the extract made of the underground part of *A.retroflexus* L. In the applied concentration of 100 g/l, deviation in maize seed epicotyls length was -1,8737 mm in comparison to control. The established value of the t-test was -2.6792, and evaluated possibility of the first grade error was 0.017158, which was less than statistical significance of the test alpha=0,05. For the applied concentrations of 25 and 100 g/l of extract made of the underground part of *A.retroflexus* L. Statistically significant difference was measured in comparison to control. Extract concentrations of 50 and 75 g/l did not show that there was statistically significant difference in comparison to control.

CONCLUSIONS

Experimental tests revealed that there was no statistically significant difference in soybean seed hypocotyls length for each separately applied extract concentration made of the above ground parts of *A.theophrasti* Med. It was established that the average hypocotyls length for the lowest concentration value of 25 g/l was 3.5524 mm lower in comparison to control variant. Statistical data processing proved that the difference in the average length of soybean seed hypocotyls increases by increase in extract concentrations. For the highest extract concentration of 100 g/l

made of the above ground parts of *A.theophrasti* Med., the established value was 5.12 mm lower in relation to control variant. The extract made of the underground part had equalized values of hypocotyls lengths in all extract variants, as well as in control. Extracts made of the above ground parts of *A.theophrasti* did not show allelophatic effect to soybean seed epicotyls growth in all studied concentrations. Extract made of the underground part of *A.theophrasti* Med. did not show statistically significant differences to the epicotyls lengths. The performed studies revealed reduced germination capability of soybean seed hypocotyls after treatment with extracts made of the above ground parts of *A.theophrasti*, which was proved by calculated statistical significance, i.e. existence of the allelophatic effect. In percentages it was established that soybean seed hypocotyls lengths in the test with application of the extract of the above ground parts of *A.theophrasti* Med. had lower values in comparison to control variant of 17-23.76%.

Based upon studies on allelophatic effect of the extract made of the above ground part of *A. retroflexus* L. that had statistically significant influence to inhibiting action on maize seed hypocotyls, it was established that increase in extract concentration results in difference in hypocotyls lengths. After application of the extract in concentration of 25 g/l, made of the above ground parts of *A. retroflexus* L., the measured average lengths of hypocotyls were lower for 1.467 mm in comparison to the average hypocotyls lengths in control variant, while application of the extract in concentration of 100 g/l the average value of hypocotyls lengths was lower in comparison to the control for 4.535 mm. Extract made of the underground part of *A. retroflexus* L. did not inhibit hypocotyls growth. However, the highest extract concentration of 100 g/l had inhibiting effect to the growth of epicotyls lengths with the average difference of 2.3244 mm.

The extract made of the underground part of *A. retroflexus* L. in the lowest concentration of 25 g/l had the lowest average epicotyls length of 1.873 mm in comparison to the control, while for the highest extract concentration of 100 g/l the largest difference in the average epicotyls length was established, which was less in relation to the control variant.

Extract made of the above ground parts of *A.retroflexus* L. showed allelophatic effect to the maize seed epicotyls growth in concentration of 100 g/l, while for concentrations of 24, 50 and 75 g/l allelophatic effect was not recorded.

After treatment by extract made of the above ground parts of *A. retroflexus* L., the reduced germination capability of maize seed was established, which was proved by values of statistically significant differences that confirmed existence of allelophatic activity. The average hypocotyls length in laboratory tests with the extract made of the above ground parts of A. retroflexus L. was lower in comparison to control for 5.04-14.53 %. The extract made of the underground part of *A.retroflexus* L. did not show allelophatic effect to the growth of maize seed hypocotyls. The obtained results confirmed that extract made of the above ground parts of *A.retroflexus* L. showed allelophatic effect only in concentration of 100 g/l. Results of the study confirmed that allelophatic activity of extracts made of the underground part of *A. retroflexus* L. showed allelophatic effect to the growth of maize seed epicotyls in the highest applied concentrations of 75 and 100 g/l of weed plant mass, in values for 22.53-28.8% lower in relation to the control variant.

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