Study on the allelopathic effect of *Amaranthus retroflexus* L., *Datura stramonium* L. and *Panicum miliaceum* L. on the germination of maize

Untersuchung zur allelopathischen Wirkung von Amaranthus retroflexus *L.,* Datura stramonium *L. and* Panicum miliaceum *L. auf die Keimung von Mais*

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Abstract

Plant extracts of *Amaranthus retroflexus* showed an inhibitory effect on the development of both cotyledons and rootlets of maize in petri dish trials. In pot experiments, among the three examined weeds, dry plant residues of *Amaranthus* hindered the germination of maize to the greatest extent. It showed also a negative effect on the shoot length and weight, while there was a positive effect on the root length and weight.

A 2.5% concentration of *Datura stramonium* plant extracts showed a stimulative effect, but the 5% and 7.5% extracts inhibited the shoot and root development of germinating maize. In spite of a 60% germination rate, the incorporation of dry plant residues into the soil did not show any significant effect on the development of shoots, but a stimulative effect on root development was observed. However, the dry weight of roots exceeded the control values only at a concentration of 7.5%.

Plant extract of *Panicum miliaceum* had a stimulative effect on the shoot and root development of maize. Dry plant parts in the soil of the pots hindered the germination of maize, but significant effects on the shoot and root lengths could not be proven. At the 7.5% ratio the dry weight of shoots was higher than the control values, but all the other soil-plant part ratios caused lower values.

Keywords: *Amaranthus retroflexus, Datura stramonium,* dry weight, germination, *Panicum miliaceum,* plant extract, root, shoot

Zusammenfassung

Der Pflanzenextrakt von *Amaranthus retroflexus* zeigte eine hemmende Wirkung auf die Entwicklung der Kotyledonen und Wurzeln von Mais in Petrischalen-Versuchen. In Topfversuchen reduzierten die Trockenpflanzenreste von *Amaranthus* die Keimung von Mais weitestgehend. Auch zeigte sich ein negativer Effekt auf Sprosslänge und -gewicht, während ein positiver Effekt auf die Wurzellänge und -gewicht deutlich wurde.

Eine 2,5 %ige Konzentration von *Datura-stramonium*-Pflanzenextrakten zeigte eine stimulierende Wirkung, aber die 5 %- und 7,5 %igen Extrakte hemmten die Spross- und Wurzelentwicklung von keimendem Mais. Trotz 60 %iger Keimungsrate zeigte die Einarbeitung von trockenen Pflanzenresten in den Boden keine signifikanten Auswirkungen auf die Sprossentwicklung, aber es wurde eine stimulierende Wirkung auf die Wurzelentwicklung beobachtet. Das Trockengewicht der Wurzeln überschritt jedoch die Kontrollwerte nur bei einer Konzentration von 7,5 %.

Der Pflanzenextrakt von *Panicum miliaceum* wirkte stimulierend auf die Spross- und Wurzelentwicklung von Mais. Die trockenen Pflanzenteile im Boden der Töpfe behinderten die Keimung von Mais, aber signifikante Effekte auf die Trieb- und Wurzellängen konnten nicht nachgewiesen werden. Bei der Konzentration von 7,5 % war das Trockengewicht der Triebe höher als die Kontrollwerte, aber alle anderen Boden-Pflanzenteil-Raten hatten niedrigere Werte.

Stichwörter: *Amaranthus retroflexus, Datura stramonium,* Keimung, *Panicum miliaceum,* Pflanzenextrakt, Trieb, Trockengewicht, Wurzel

Introduction

One of the solid bases of today's agricultural production is the effective control of weeds. Weeds are responsible for about 34% of yield losses globally. Moreover, resistant species are revealed increasingly (KHAWAR et al., 2015). Improperly timed weed control measures, repetitive use of similar herbicides and spraying at an inappropriate phenological stage under unfavourable meteorological conditions can accelerate this process (SOLYMOSI, 1990). There are about 6700 weed species having effects on the plant production worldwide, 200 of them can be considered important, which may cause serious damage on every site where agricultural production is taking place (HOLM et al., 1977). Modern weed control has to be performed in the spirit of integrated plant protection, which means that efforts should be made to ensure properly timed mechanical weed control, profound knowledge of the weed flora and the prediction of the expected weed coverage of the given field.

Based on these, the number of treatments with herbicides should be reduced as far as possible and combination of differently acting herbicides should be used to avoid the appearance of resistant or tolerant biotypes of weeds. The headway of herbicide resistance requires more and more active ingredients, but their number is finite, and in addition to it, currently used herbicides are not allowed in biological production (SOLTYS et al., 2013). To eliminate this factor, it is important to have as many tools and technologies as possible to reduce the damage to weeds.

Understanding allelopathy helps to understand the interaction between cultivated plants and the weed flora and it may open up new opportunities for integrated and biological plant protection. The key compounds of allelopathy are the so-called allelochemicals that are synthesized by plants having allelopathic effects. These biologically active compounds have effects on the development of other plants in the surrounding of the emitting plant. Most of these compounds have negative effects, but they may have positive effects as well. These compounds can cause disturbance of germination or growth by upsetting photosynthesis, respiration, water balance and hormonal equilibrium, which processes are typically based on enzyme inhibitions (RICE, 1974, 1979, 1984; PUTNAM and TANG, 1986; SZABÓ, 1994; SOLYMOSI, 1996; SZABÓ, 1997; INDERJIT and KEATING, 1999). By studying allelochemicals, exploring their mode of action, based on allelopathic processes, even new active ingredients can be produced, which can be used later in weed control. All these help to avoid the resistance of weeds and reduce the load of the environment with herbicides.

The use of allelopathic compounds can be an important component of future integrated and environmentally sound weed management, as increasing emphasis is placed on the research, development and application of environmentally friendly plant protection methods and devices.

In addition to the traditional chemical protection, integrated plant protection is preferred and the research of natural substances that can be used in organic and biological farming is also emerging.

Materials and Methods

Green samples of the given weed plants were collected in the summer of 2015. The samples of *Amaranthus retroflexus* and *Datura stramonium* were collected in August from sunflower and maize fields at Bajót (Komárom-Esztergom county, Hungary), the samples of *Panicum miliaceum* were collected from a stubble field in the vicinity of Keszthely (Zala county, Hungary).

The collected plant material, purified from soil residues has been dried for 6 weeks until air-dry condition. At the end of the drying process, a predetermined portion of the samples was selected for the Petri dish trials, and the rest was kept for the second stage of the experiment series for pot experiments. The dried plant parts (shoots, leaves) were cut into 1-2 cm pieces with secateurs and later homogenized with a coffee grinder.

The purpose of this experiment was to prove the allelopathic effect of the given species.

The experiment was designed with triple dilution line (2.5%, 5% and 7.5%) of plant extracts; therefore dry plant material was measured in required quantities for 200 ml of liquid, in this case distilled water, to obtain the desired solution. Plant samples had been left in the water within measuring cups for 24 hours at room temperature to allow the allelochemicals to dissolve.

At the end of the dissolution, Petri dishes were prepared. The seeds were placed on double-layer filter paper, and then were poured with 15 ml of extract (distilled water for control). For each concentration, 8 replications were used.

The germination experiment was performed with the seeds of the maize variety 'MV Koppány', placing 25 pieces in each cup. As a control, 4 replications were made, using only water instead of plant extract.

Subsequently, the Petri dishes were kept for 7 days at 20°C in a germinating incubator. The moisture content lost during the incubation was retrieved with water spray.

As an evaluation of the experiment, the germinating seeds were individually examined on the 7th day.

By measuring the length of the cotyledons and rootlets 2 times, 200 length data for each extraction concentration were taken. The average of these data was considered as the result of the respective concentration. The same method was used in the case of control Petri dishes, where the average length of 100 cotyledons and rootlets were calculated.

This process was carried out in the same way for all 3 weed species. The evaluation of seedlings treated with the extract of *Amaranthus retroflexus* was carried out on 11.04.2015, with the extract of *Datura stramonium* on 11.11.2015 and with the extract of *Panicum miliaceum* on 18.11.2015.

Pot experiment to study the effects of shoot and leaf residues of *Amaranthus retroflexus*, *Datura stramonium* and *Panicum miliaceum* on the germination of maize were also carried out.

In the second part of the experiment, the remainder part of the weed samples collected for the previous experiment were used, so their collection time and place were the same. In this case, however, 1-2 cm of dried plant pieces were broken into a smaller size range of 1-6 mm by an electric coffee grinder.

Two litres of common black mould were put into the pots, which served as a nutrient medium (Fig. 4). To the given medium, the 2.5%, 5% and 7.5% dilution line was used, adding the plant debris required for the desired concentration. The shredded plant parts were incorporated into the soil within the upper 6 cm layer.

For each concentration, 4 replications were planned, so for each mixture of 2.5% and 5% and 7.5% soil-plant parts 4 pots were assigned with 5 maize kernels per pot. The kernels were sown in a depth of 3 cm. For the control also 4 replications were designed; there were only black mould and maize kernels in the control pots.

The 4 pots belonging to the certain concentrations, with seeds and weed residues after being injected with 200 ml of water, were placed into a BINDER-type rearing chamber at 20°C for 30 days. The constant moisture was kept by irrigation throughout the germination period.

Fig. 1 Maize plants derived from pots treated with the residues of *Datura stramonium.*

***Abb. 1** Maispflanzen aus Töpfen, die mit den Resten von Datura stramonium behandelt wurden.*

The evaluation took place after 30 days. The germination percentage according to certain concentrations was calculated and the dry weight and the length of roots and shoots were measured per plant. The average of the repetitions of the same plant residues / soil ratio was taken to the end result of the experiment.

All these steps were performed separately for each of the 3 weed species tested. The evaluation of maize treated with *Amaranthus retroflexus* samples was carried out on 19.10.2019, with *Datura stramonium* samples on 27.01.2017 (Fig. 1) and with *Panicum miliaceum* samples on 28.02.2017.

Results

Results of the Petri dish germination trials carried out with the plant extracts from *Amaranthus retroflexus, Datura stramonium* and *Panicum miliaceum*

In the case of all 3 plant extracts, the germination percentage ranged from 99% to 100% in every replication and in all concentrations, so a detrimental effect of the extracts on the germination of maize could not be revealed. Each seed developed a cotyledon and rootlet. The length of these cotyledons and rootlets was compared with the results of the control group to ascertain whether the extracts of weeds have an allelopathic effect on the germination.

During the evaluation, it was observed from the measured data that *Amaranthus retroflexus* extracts caused shorter lengths in the case of both the cotyledons and rootlets. Under the 2.5% dilution, the cotyledons reached 67% of the control average size, which was 25% in the case of the 5% dilution while it was 45.56% in the case of the 7.5% dilution. Thus, the strongest inhibition of shoot development was induced by the 5% solution.

Rootlets were also less developed in comparison to the control; in this case, the detrimental effect was proportional to the concentrations so the strength of inhibitory activity ranged from the smallest concentration to the highest. In reaction to the 2.5% plant extract, the rootlets reached 97% of the control size, 83.24% in the case of the 5% and 74.73% in the case of the 7.5% extracts.

Datura stramonium extracts also had an inhibitory effect. The average size of the cotyledons decreased proportionately with the concentration of the plant extracts, reaching 52.11% of the control size in the case of the 7.5% dilution. Rootlets showed a transient stimulatory effect at a concentration of 2.5% with more developed root components than the control ones, but in the case of 5% and 7.5% extracts growth inhibitory effect was manifested.

In the case of *Panicum miliaceum*, a stimulative effect was observed, similarly to the previous results of PRUTENSKAYA (1974). The 5% concentration had the highest positive effect on the growth of cotyledons; in this case the average length of them was 56.92% higher than the mean values of the control. The same positive effect was observed on root growth, where the average root size was 29.88% higher than the average root size.

By comparing the data by using single-factor analysis of variance, it can be established that there was a difference at 5% significance level between the effect of each weed extract on rootlets and cotyledons compared to controls and concentrations. These were potent inhibitory effects in the case of *Amaranthus retroflexus* and *Datura stramonium* while stimulative effect in the case of *Panicum miliaceum*.

Results of groups by concentrations with their significance level are shown in Table 1.

Tab. 1 Results of the Petri dish germination trials.

Tab.1 *Ergebnisse der Petrischalen-Keimungsversuche.*

Weed extract	Germinating maize	Control	2.5%	5%	7.5%	SD5%
Amaranthus retroflexus	Length of cotyledon (mm)	21.30	14.43	5.33	9.73	21.30
	Length of rootlet (mm)	89.35	86.85	74.38	66.78	7.68
Datura stramonium	Length of cotyledon (mm)	21.3	34.05	19.76	11.10	2.59
	Length of rootlet (mm)	89.35	98.09	63.61	57.19	5.99
Panicum miliaceum	Length of cotyledon (mm)	21.30	33.43	44.03	25.38	4.37
	Length of rootlet (mm)	89.35	105.13	116.05	99.55	7.49

Group results of the length of cotyledons and rootlets compared to the control are demonstrated in Figure 2.

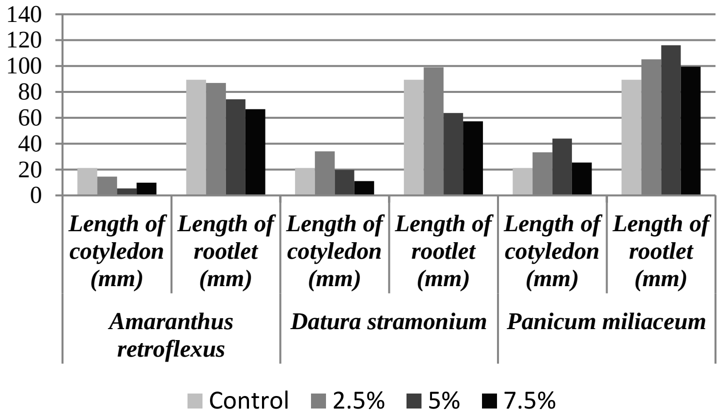

Fig. 2 Overall results of the Petri dish trials.

Abb. 2 *Gesamtergebnis der Petrischalenversuche.*

It can be seen that the inhibitory or stimulative effect does not necessarily follow the dilution line. The 2.5% solution of *Datura stramonium* had a stimulative effect, only the more concentrated solutions showed inhibitory effect. In the case of the *Panicum miliaceum* extracts, the 5% concentration showed the strongest stimulative effect. The reason of this may be that allelochemicals are able to quench the effect of excessive nutrients in the solution at a given concentration. In addition, consideration should be given to the inhibitory effects derived from the potential differences of solutions, which are independent from the allelopathic effects.

The more concentrated solutions are more difficult to absorb into the seeds, so they can absorb less water for germination. Additionally, plant residues contain spare nutrients besides allelochemicals, which may be released and become available for germinating seeds as germination supporting agents. These effects make it difficult to study allelochemicals.

Results of the pot experiment carried out with stalk and leaf residues of *Amaranthus retroflexus*, *Datura stramonium* and *Panicum miliaceum* on the germination of maize

Germination percentage

In the pot experiment, the effect of weed shoots and leaf fragments on maize germination appeared detrimentally as compared to the control group. In the control pots, all maize seeds germinated successfully, resulting in 100% germination.

During the experiment, it was revealed that the germination of maize was reduced in the case of all weeds in all concentrations compared to the control (Fig. 3). In the greatest extent, *Amaranthus retroflexus* plant parts hindered the germination, but reversely to the proportion of plant debris, the smallest concentration inhibited mostly the germination of maize (35% germination).

Datura stramonium also decreased the germination, but soil-plant residue ratios did not show any significant difference in this extent. In all three concentrations, the germination ratio was about 60%.

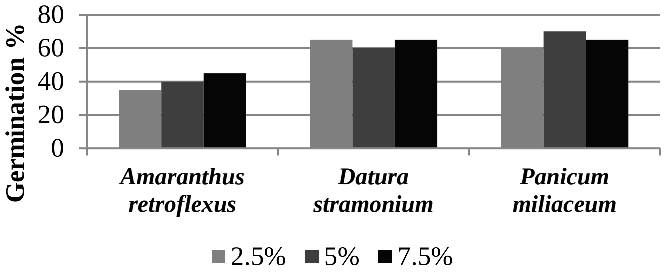

Fig. 3 Germination percentage results for the maize pot experiment according to the concentration of weed plant residues.

Abb. 3 *Prozentuale Keimung von Mais im Topfversuch in Abhängigkeit der Konzentration der Unkrautpflanzenreste.*

Panicum miliaceum plant parts also had a hindering effect on the germination compared to the control group. In this case, the 5% soil-residue ratio resulted in the highest plant number, similarly to the Petri dish experiment, where the 5% solution had the highest stimulative effect (Fig. 3).

Effect of weed plant residues on the length of roots and shoots

In this experiment, shoot and leaf residues decomposed during germination, so besides the allelopathic effect, the nitrogen and water removal effect of the bacteria involved in the decomposition has to be taken into account as well. The absence of germination and differences in cotyledon and rootlet lengths may be attributed to this effect, apart from the effects of any allelochemicals.

During the evaluation, the length of shoots and roots were measured separately. Their lengths were compared to the results of the control group in order to determine whether any inhibitory or stimulative allelopathic effect on germinating maize's shoot and root lengths manifested on the effect of weed shoot and leaf residues incorporated into the medium. The data were processed by single-factor analysis of variance.

After comparing the results, it was found that *Amaranthus retroflexus* caused the smallest germination percentage, but the greater the amount of its plant residues in the pots, the greater the length of roots and shoots developed on the maize (Tab. 2). At a 7.5% plant part ratio, the average length of the shoots corresponded to the average length of the control. The average length of the roots exceeded the control group's values with the exception of the 2.5% concentration. Both the shoot (SD5% = 7.21%) and the root growth (SD5% = 8.81%) showed significant differences compared to the values in the control group.

In the case of *Datura stramonium*, although the average length of maize shoots decreased proportionally with the concentrations, it did not show significant differences (Tab. 2). The root length averages (SD5% = 9.83) however differed significantly. In this case, the highest root mass was developed at the 5% concentration, but the root mass at all concentrations exceeded its values in the control group, so a kind of stimulative effect occurred.

In the case of *Panicum miliaceum*, no significant difference between the shoot (SD5% = 9.04) and the root lengths (SD5% = 9.01) was found compared to the control group. The results are shown in Table 2.

Tab. 2 Shoot and root length results of germinating maize in the pot experiment according to the soil-plant part ratios (%).

Tab. 2 *Spross- und Wurzel-Längen von keimendem Mais im Topf-Experiment je nach den Boden-Pflanzenteil-Verhältnissen (%).*

Weed species	Germinating maize	Control	2.5%	5%	7.5%	SD5%
Amaranthus retroflexus	Length of shoot (cm)	54.55	34.43	42.25	54.78	7.22
	Length of root (cm)	37,40	35.00	56.63	46.22	6.82
Datura stramonium	Length of shoot (cm)	54.55	55.62	51.25	50.62	10.25
	Length of root (cm)	37.40	49.77	51.25	41.69	9.83
Panicum miliaceum	Length of shoot (cm)	54.55	48.17	44.86	46.08	9.05
	Length of root (cm)	37.40	40.42	36.00	33.85	9.01

The average length of shoots and roots according to the experimental groups and concentrations are shown in Figure 4.

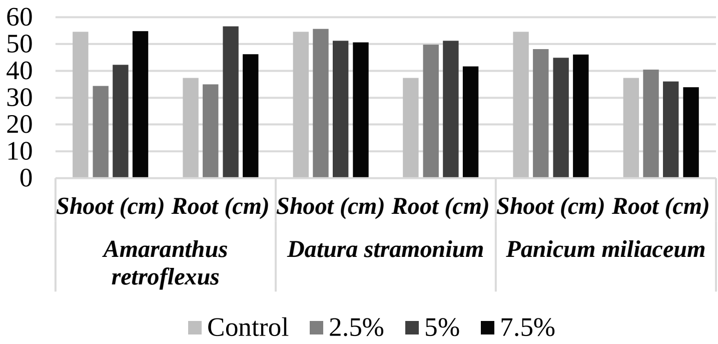

Fig. 4 Length of shoots and roots of maize according to the soil-plant ratios.

Abb. 4 *Länge der Sprossen und Wurzeln von Mais in Abhängigkeit des Boden-Pflanzen-Verhältnisses.*

Effect on the dry weight of maize plants

The germinated maize plants were weighed and left to become dry. The dry weight of roots and shoots were measured separately. From the total weight and number of germinated plants, the average dry weight of experimental groups per plant could be calculated. So our results show the average dry weight per plant. The values found during the measurement are summarized in Table 3.

Under the effect of *Amaranthus retroflexus* plant debris, the dry weight of maize plants lagged behind the control group. The lowest dry weight value was measured at a ratio of 2.5% and the dry weight per plant was lagged to the highest extent in comparison with the control. The inhibitory effect was inversely affected by the increase in concentration, and at 7.5%, the dry weight almost reached the control value. The dry weight of roots at all three concentrations exceeded the control group's values by far, which fits to the stimulative effect on the root lengths. Therefore, *Amaranthus retroflexus* had a positive impact on the growth of the roots.

Tab. 3 Dry weight of maize shoots and roots according to the experimental groups and weed species in the pot experiment.

Tab. 3 *Trockengewicht der Sprosse und Wurzeln von Mais in Abhängigkeit der experimentellen Gruppen und Unkrautarten im Topfversuch.*

Weed species	Dry weight/plant specimen	Control	2.5%	5%	7.5%
Amaranthus retroflexus	Shoot (g)	0.5	0.19	0.44	0.47
	Root (g)	0.31	0.62	0.68	0.67
Datura stramonium	Shoot (g)	0.50	0.60	0.45	0.65
	Root (g)	0.31	0.22	0.28	0.32
Panicum miliaceum	Shoot (g)	0.50	0.36	0.39	0.52
	Root (g)	0.31	0.15	0.23	0.26

Under the effect of *Datura stramonium* plant residues, with the exception of the 5% ratio, both groups showed a stimulative effect on the dry weight of the shoot. The root weight remained below the control values at 2.5% and 5%, but exceeded them in the 7.5% group.

Panicum miliaceum had a negative effect on both the root and shoot weight. Only 7.5% concentration resulted in a higher shoot weight ratio than the control group (Fig. 5).

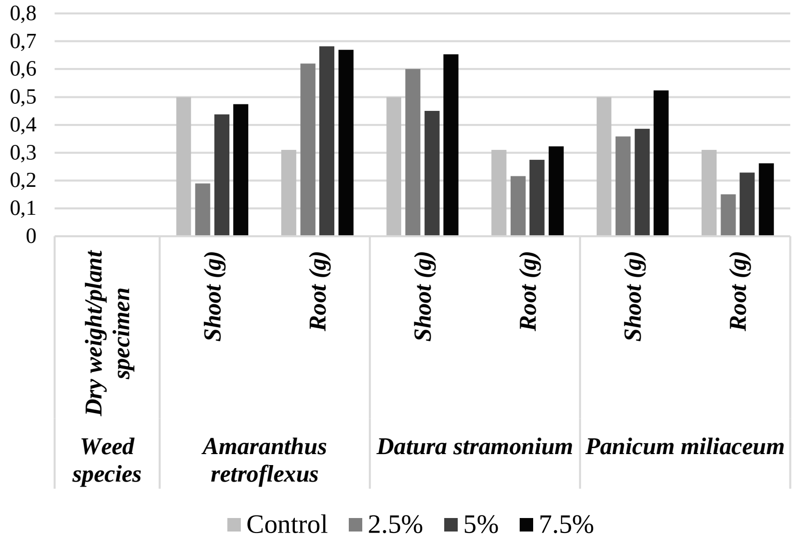

Fig. 5 Dry weight of maize shoots and roots under the effect of different ratios of weed stalk and leaf residues in the pot experiment.

Abb. 5 *Trockengewicht-Daten von Mais-Trieben und -Wurzeln in Abhängigkeit der verschiedenen Verhältnisse von Unkraut Stiel- und Blattresten in den Topf-Experimenten.*

Conclusions

A number of research studies have proved that some weeds have allelopathic effects that affect the development of other plants in their surroundings. The three species, *Amaranthus retroflexus*, *Datura stramonium* and *Panicum miliaceum* studied in this research based on previous results, also belong to these group based experiments of previous years and decades. In addition to their allelopathic effect, they are considered as major weeds. According to the latest (2007-2008) national weed survey, *Amaranthus retroflexus* is the 7th, *Datura stramonium* is 9th, and *Panicum miliaceum* 10th in the list of the most dangerous agricultural weeds. In the light of this, knowledge of allelopathic effects of these weeds is important for successful weed control and for prevention of their further spread.

Petri dish germination trials

The *Amaranthus retroflexus* extract showed growth inhibitory effect on both the maize cotyledons and rootlets. The effect on root development closely followed the elevation in the concentrations so the highest concentration caused the strongest inhibition. *Amaranthus retroflexus* plant extracts therefore exhibit a negative allelopathic effect on maize.

In the case of *Datura stramonium* extracts, the smallest, 2.5% concentration caused a stimulative effect on both cotyledons and rootlets, but the 5% to 7.5% solutions had inhibitory effects.

In the case of *Panicum miliaceum*, a stimulative effect was observed for the growth of both cotyledons and rootlets in each of the 3 concentrations. The 5% solution exerted a stimulative effect to the greatest extent. *Panicum miliaceum* extracts showed allelopathic effects on germination, as PRUTENSKAYA (1974) had shown earlier.

By evaluating the data with single-factor analysis of variance, it can be stated that at 5% significance level there was a difference among the effects of all weed extracts on cotyledon and rootlet development of maize, so these results are statistically verified.

From the above results, it can be seen that *Amaranthus retroflexus* and *Datura stramonium* are also a major competitor in the cultivation environment of maize. Not only by their competitive properties but also by their germination inhibitory effect they can make it difficult for maize to germinate and develop. Both weed species produce allelochemicals that can inhibit the germination and subsequent development of crop plants.

Although *Panicum miliaceum* is a major weed, causing serious damage its plant extract has a stimulative effect on the germination and later shoot and root developments of maize.

Pot experiments

When incorporating *Amaranthus retroflexus* shoot and leaf residues into the soil, the average shoot and root lengths of germinating maize differed from the control group. The 2.5% and 5% plant-soil ratio had a negative effect on the germination of maize. In the case of root length, the 5% and 7.5% ratios showed a stimulative effect. For each of the 3 concentrations, the dry mass of root per plant was higher than that in the control group, however, the dry weight of shoots per plant was below the control values.

The *Datura stramonium* plant parts had a stimulative effect on the root development of maize, all three ratios showed similar effect, with the 5% group being most prominent. The effect of residues of *Datura stramonium* on the shoot and root length could not be statistically proven, there was no significant difference among groups.

The groups treated with the residues of *Panicum miliaceum* did not show any significant difference the stimulative or inhibitory effects in this experiment were not statistically proven.

The dry plant parts of all three weeds resulted in a decrease in germination percentage in all tested concentrations compared to the control.

A number of factors that are important to consider can complicate allelopathic examinations. The concentration of the plant extracts with the growth of the osmotic potential can in itself have an inhibitory effect besides the effects of allelopathic compounds. Decomposing plant residues remove nitrogen and water from the soil, which also has a detrimental effect on the development of the young plants independently on allelopathic effects.

Investigations should consider these effects and it is important to perform the trials primarily in combinations. More accurate and reliable results can be obtained by combining laboratory tests (bioassays, greenhouse and field experiments) to investigate the allelopathic effects of certain plants.

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