Effect of Herbicides Paraquat and Glyphosate on the Early Development of Two Tested Plants

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Abstract. The publication deals with the effect of herbicides paraquat (PQ) and glyphosate (G) on germination and early development of standard test plants – garden cress (*Lepidium sativum* L.) and radish (*Raphanus sativus* var. *radiculata* L.). PQ has proven environmental toxicity and its usage is forbidden in Europe, while G is widely used in agricultural practice. Pollution of soil and surface water with pesticides can cause a reduction in biodiversity and species abundance, alteration in the structure of populations with consequent degradation of terrestrial communities. Besides their ability to bioaccumulate and biomagnify along the trophic chains and thus remain in the biotope over a long time in increasing concentrations, they can inhibit seed germination and early development of young plants in ecosystems. The ecotoxicology tests were conducted with 200 µM, 350 µM, 500 µM, 650 µM, 800 µM, 950 µM and 1100 µM herbicides concentrations, and a control – distilled water. The number of germinated seeds (Ek) and viable sprouts (K), length of stems and roots, and absolute dry weight of stems, roots and leaves were used as parameters for identifying the pesticides impact. The ecotoxicology tests showed a generally pronounced higher toxicity of PQ compared to G. In addition, the genetic analysis using ISSR markers showed that plants respond to herbicide stress through changes at DNA level that are in general dose-dependent and, at least partially, stress-specific.

1. Introduction

During the past decades, population growth has become a challenge for agriculture in order to supply the human needs for food [1]. On the other hand, pests, pathogens [2], weeds and unfavorable climate changes [1] decrease the amount of produced crops. In order to cope with this problem, new pesticides are used for plant protection [2] and hence global crop production has increased [1]. The herbicides do not only control target weeds, but also non-target organisms [3] as well as many authors report that their residues in soil can have ecologically negative impact [4-7]. For example, Pimentel and Levitan [8] point out that pesticides are a strong source of environmental pollution as 2.5 million tons of pesticides worldwide are used yearly, 99.9% of which moves into the environment without reaching the target pests.

The herbicides represent 50–60% of pesticides used [9] as paraquat, PQ (or methyl viologen; N,N9-dimethyl-4,49-bipyridinium dichloride) [10, 11] and glyphosate, G (N-(phosphonomethyl)-glycine) are one of the most widely used nonselective herbicides [12].

PQ is used for the control of broadleaf weeds due to its great efficiency and low cost [13]. PQ is adsorbed very quickly by plant leaves and blocks photosynthesis by accepting electrons from photosystem I (PSI) [3]. This prevents the formation of NADPH [14] and reactive oxygen species are formed [14-16]. This leads to the formation of superoxide anions, single oxygen, and hydroxyl and peroxyl radicals in chloroplasts [17], which attack biomembranes [18]. According to the published information, PQ becomes biologically inactive in soil and has minimal or no toxicity toward roots and rhizomes. In addition, PQ has no effects on mature bark [15, 19]. Because of these characteristics, PQ was used in orchards, plantation crops, conservation tillage systems, and other applications [20, 21].

Glyphosate controls most annual and perennial weeds [22] and it is widely used in agriculture, forestry, landscape management for removal of undesirable vegetation from aquatic and urban ecosystems [23] like roadsides, irrigation channels, recreational areas, and for woody weed control [24]. It is also applied for burndown and postemergence applications in G-resistant transgenic crops [12, 25-27]. Glyphosate inhibits growth by causing chlorosis at the newest growing points and necrosis throughout the entire plant [28]. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimate pathway [29] as the plant is unable to produce the aromatic amino acids phenylalanine, tryptophan, and tyrosine needed for growth [27, 28, 30, 31].

The aim of the research is to compare the influence of herbicides PQ and G on the germination and early development of standard test plants in ecotoxicology – garden cress (*Lepidium sativum* L.) and radish (*Raphanus sativus var. radiculata* L.). The two pesticides were selected, because of the differences in usage: PQ is banned in EC and G is still widely applied in agronomy. This will give insights about the possible effects of G over usage.

2. Materials and Methods

The *Lepidium sativum* L. seeds (Lot No ST 138293, Italy), and *Raphanus satuvus* var. radiculata L. seeds were sorted by size and color and left in a refrigerator for 24 h, before attempting to practice, as instructed by Bulgarian Seed Standard [32-34].

2.1. Setting up the tests

2.1.1. Processing the Petri dishes

Petri dishes were washed with dH_2O, dried and treated with 96% ethyl alcohol. Filtering paper was placed in each of the dishes (2 layers on the plates and 1 layer on the lid) and then autoclaved under dry steam for 30 min at 1 atm (121° C).

2.1.2. Setting up the samples

The seeds of garden cress and radish (150 seeds per plant and per every concentration and control) were treated with different concentrations of PQ and G (200 μM, 350 μM, 500 μM, 650 μM, 800 μM, 950 μM and 1100 μM) and a control sample with dH_2O [34]. The seeds were evenly distributed over the germinate bed and possibly spaced apart from each other to avoid touching the germinated seeds each other before enumeration and removal (according to BSS 601-85). The two-layer filter paper of the plate was moistened with 10 ml, and the single layer of the lid - with 5 ml of the respective herbicide concentrations and dH_2O for the control. On the second and third day respectively, 4 ml of the appropriate concentrations and dH_2O was added to the germination bed– 2 ml to the plate and 2 ml to the lid. The cultivation was performed at 55% humidity and 23.9°C (BSS 601-85 requirement 20-30° C). The temperature and humidity were recorded with a thermo-hygrometer (TFA Dostmann Ltd., Germany).

2.1.3. Parameters measurement

The measured physiological parameters were: germinating energy (Ek), germination (K) of the seeds, length of root (Lr) and stem (Ls), as well the biomass of garden cress and radish sprouts. The Ek was reported by counting germinated seeds on the 4th day. The number of vital germinated seeds (K) was

counted respectively on the 6th day for radish and the 10th day for garden cress from the test starting [32]. The root and stem lengths were recorded by measuring each individual [33, 35], then the roots, stem and leaf mass were removed, dried at 85°C for 48 hours, and the absolute dry weight was determined respectively in the treatment of both types of herbicides at different concentrations [34]. The parameters: LC_{50} - the lethal concentration cased death for 50% of tested seeds and sprouts (for Ek and K) against the control as well as EC_{50} – the effective concentration cased in average 50% decreasing of the lengths (for Lr and Ls) against the control were calculated [36, 37].

2.2. Genetic analysis

The ability of the herbicides used to induce DNA mutations was studied using ISSR markers. Samples from treated and non-treated *Raphanus sativus var.radiculata* L. plants with pesticides concentration of 500 μM PQ and 950 μM G without manifested infections were selected.

2.2.1. DNA *extraction*

DNA extraction was done from 15-20 plants/treatment using DNA Extraction Kit Phytopure™ (GE Healthcare UK Limited). The concentration and quality of DNA were checked by gel electrophoresis on 1% agarose gel and by measurement on Nanodrop 2000 spectrophotometer (Termo Scientific).

2.2.2. Polymerase Chain Reaction

Inter-Simple Sequence Repeat technique (ISSR) with 10 primers (8 dinucleotide and 2 tri-nucleotide) amplifying different genomic regions were used for PCR (Table 1). PCR was performed in a 25 μl reaction containing 12.5 μl x MyTaq™ HS Mix (BIOLINE), 2 μl DNA (15-20 ng), 0.3 μM ISSR primer and sterile mQH20.

Table 1. ISSR primer sequence information and the temperature of annealing used in PCR reactions.

Primer	Primer sequence	Temperature of annealing of primers (Ta ˚C)
ISSR1	(CT)8GC	56
ISSR2	(CA)8G	56
ISSR3	(CT)8AC	56
ISSR4	(TC)8C	55
ISSR5	(CT)9G	58
ISSR6	(AC)8G	58
ISSR7	(GA)9C	58
ISSR8	(GT)6GG	58
ISSR9	(CAC)7T	-
ISSR10	(CAC)7G	-

PCR amplification was performed on Verity 96 well thermal cycler (Applied Biosciences) under the following conditions: initial denaturation at 94 °C for 60 sec followed by 35 cycles, each comprising: 94 °C - 30 sec; Ta˚C (depending on the primer) - 60 sec; 72 ˚C - 90 sec; and final step - elongation at 72 ˚C for 10 min. Electrophoresis of amplification products was done on a 2.0% agarose gel at 100V for 1.5 hours. The software product Launch Vision Works LS was used to visualize and report the ISSR profiles.

3. Statistical analysis

The software Statistica 7.0 [38, 39] was used to find relationships and differences between variables. Correlations between the plant parameters K, Ek, Lr and Ls, and the herbicides paraquat and

glyphosate were analyzed by parametric Pearson's correlation tests (scatterplots figures). Regression equations were calculated for applied linear or polynomial regressions of dependent variables (plant parameters) impacted by herbicides. Levene's test for homogeneity of variances was applied before the analyses. The differences in variances (comparison of least squares means ± 0.95 confidence intervals) of each dependent variable under the treatment of different concentrations of the same two herbicides were tested using univariate analyses of variance (ANOVA, f-tests within groups), but presented mixed for G and PQ or for Lr and Ls in mean plots ANOVA figures. Two-way ANOVA with post-hoc Fisher's tests were performed to find the differences in the means of dependent variables between two groups (glyphosate and paraquat treatments). P-value less than 0.05 was considered statistically significant (*p<0.05, **p<0.01, ***p<0.001).

3. Results and discussions

3.1. Effects of paraquat and glyphosate on Ek and K

3.1.1. Garden cress seeds

PQ caused decrease in Ek of garden cress seeds with increasing of its concentrations - from 98.66% at 200 µM to 26% at the highest tested concentration of 1100 µM (Figure 1a). As LC_{50} for Ek, the herbicide concentration 958.99 µM was determined. The number of viable sprouts (K) decreased by approximately 50% (LC_{50}) at the concentration of 556.35 µM PQ. At the highest concentration K = 0 %, e.g. 100% lethality was reported (Figure 1b). The dependences between Ek, K and tested concentrations of PQ were linear functions (Figure 1a and 1b).

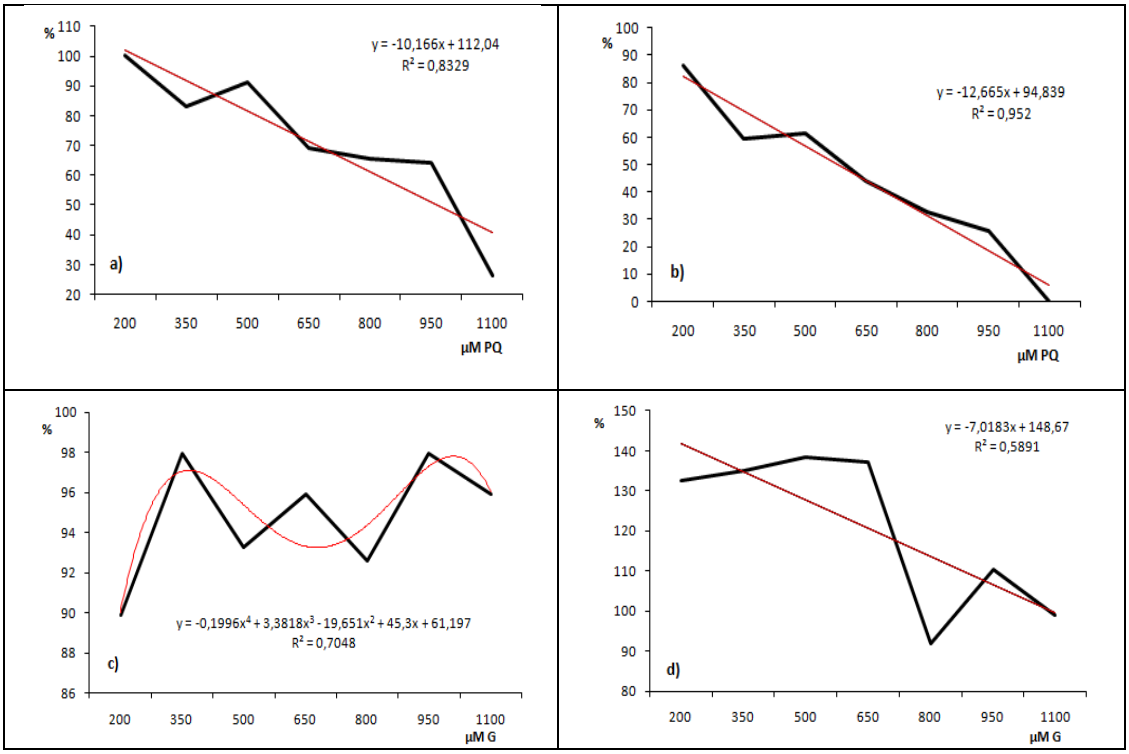

Figure 1. Ek and K of the garden cress seeds treated with PQ and G (expressed as a % of the control): a) Ek with PQ treatment, b) K with PQ treatment, c) Ek with G treatment, d) K with G treatment.

When treating the garden cress seeds with the same concentrations of G, Ek was close to that of the control, especially at concentration of 350 µM and 950 µM (Figure 1c). G at these concentrations did not have a significant effect on Ek. K showed that the pesticide had even stimulating effect except at a

concentration of 800 μM (Figure 1d). EC_{50} for Ek was 958.99 μM and for K - 556.35 μM, so the negative effect is getting stronger with longer exposure on G. The dependences between Ek, K and tested concentrations of G were respectively polynomial of four degrees and linear (Figure 1c and 1d). The observed negative effect on Ek and K of garden cress was more pronounced regarding PQ compared to G.

3.1.2. Radish seeds

Ek and K values of radish seeds treated with PQ showed fluctuations of 20% between each treatment at low concentrations (200 μM, 350 μM and 500 μM), and a gradual reduction with the increasing of PQ concentrations (Figure 2a). The similar were the observed results for K, taking into account the sprouts vitality. The toxicity was also more pronounced at the last two high concentrations (Figure 2b). PQ had more pronounced influence of on K, compared to Ek as the calculated EC_{50} was 764.2 μM and for Ek was even higher than the highest applied concentration (1324.47 μM). The dependences between Ek, K and tested concentrations of PQ were linear functions (Figure 2a and 2b).

The G treatment on Ek of radish seeds coased the light fluctuating inhibition. At concentration 500 μM, Ek had minimal value of 70% (Figure 2c). Inhibition was better expressed in the longer duration of treatment - on K (Figure 3d). A percentage close to the control was again observed up to 650 μM herbicide concentrations, and then higher mortality rates increased with increasing the G concentrations. The dependences between Ek, K and tested concentrations of G were respectively polynomial of 6^{th} degree and linear functions (Figure 2c and 2d).

The tested pesticides had an inhibitory effect on the radish Ek and K, which was statistically proven, with the exception of the influence of G on Ek. The significant to strong negative correlations for Ek and K with increasing PQ concentrations and strong correlations - for K treated with G in different concentrations were observed (Figure 3b).

The garden cress showed higher sensitivity to PQ than the radish, when comparing the values of EC_{50} for Ek and K. The opposite effect is observed, when the test objects are treated with G. In that case the more sensitive was the radish with EC_{50} for K=1333.96 μM compared to the garden cress (EC_{50} for K=2209.27 μM, higher than the highest applied concentration). For both, the garden cress and the radish, the negative effect of the pesticides on Ek were described by polynomial equations and localized at certain concentrations (Figure 1c and 2c).

The declines in seed germination rate have been reported in the literature with other pesticides; likewise, endosulfan showed a significant decrease in tomato seeds germination rate (Fatiha and Fouad 2011), paraquat dichloride decreased significantly the seed germination rate in *Typha latifolia* (Moore et al. 1999); DDT induced the inhibition of seed germination in peanut (*Arachis hypogaea*) and mustard (*Brassica juncea*) seeds (Mitra and Raghu 1989).

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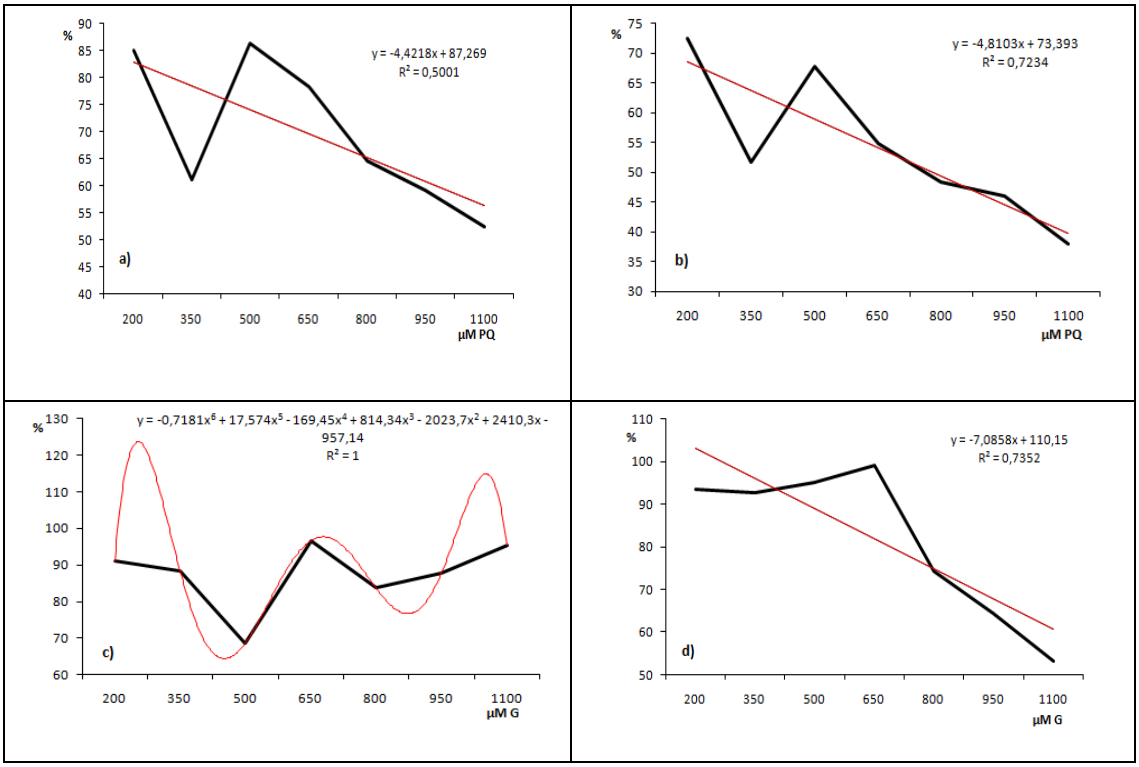

Figure 2. Ek and K of the radish seeds treated with PQ and G (expressed as % of the control): a) Ek with PQ treatment, b) K with PQ treatment, c) Ek with G treatment, d) K with G treatment.

The tested pesticides had an inhibitory effect on the garden cress Ek and K, which was not statistically proven only for the influence of G on Ek. The toxicity of PQ on Ek and K of seeds at the concentrations used was greater (the strong to very strong negative correlations) than G influence - significant negative correlation only for K (Figure 3).

The garden cress showed higher sensitivity to PQ than the radish, when comparing the values of EC_{50} for Ek and K. The opposite effect is observed, when the test objects are treated with G. In that case the more sensitive was the radish with EC_{50} for K=1333.96 µM compared to the garden cress (EC_{50} for K=2209.27 µM, higher than the highest applied concentration). For both, the garden cress and the radish, the negative effect of the pesticides on Ek were described by polynomial equations and localized at certain concentrations (Figure 1c and 2c).

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3.2. Effects of paraquat and glyphosate on the stem and root length (% of the control)

3.2.1. Garden cress

The length of garden cress stems and roots decreased with increasing PQ concentration (Figure 4a and 4b). PQ exerted stronger effect on stems length (EC_{50}=631.47 µM) compared to that of the roots (EC_{50}=747.06 µM). The dependences between Lr, Ls and tested concentrations of PQ were linear functions (Figure 4a and 4b).

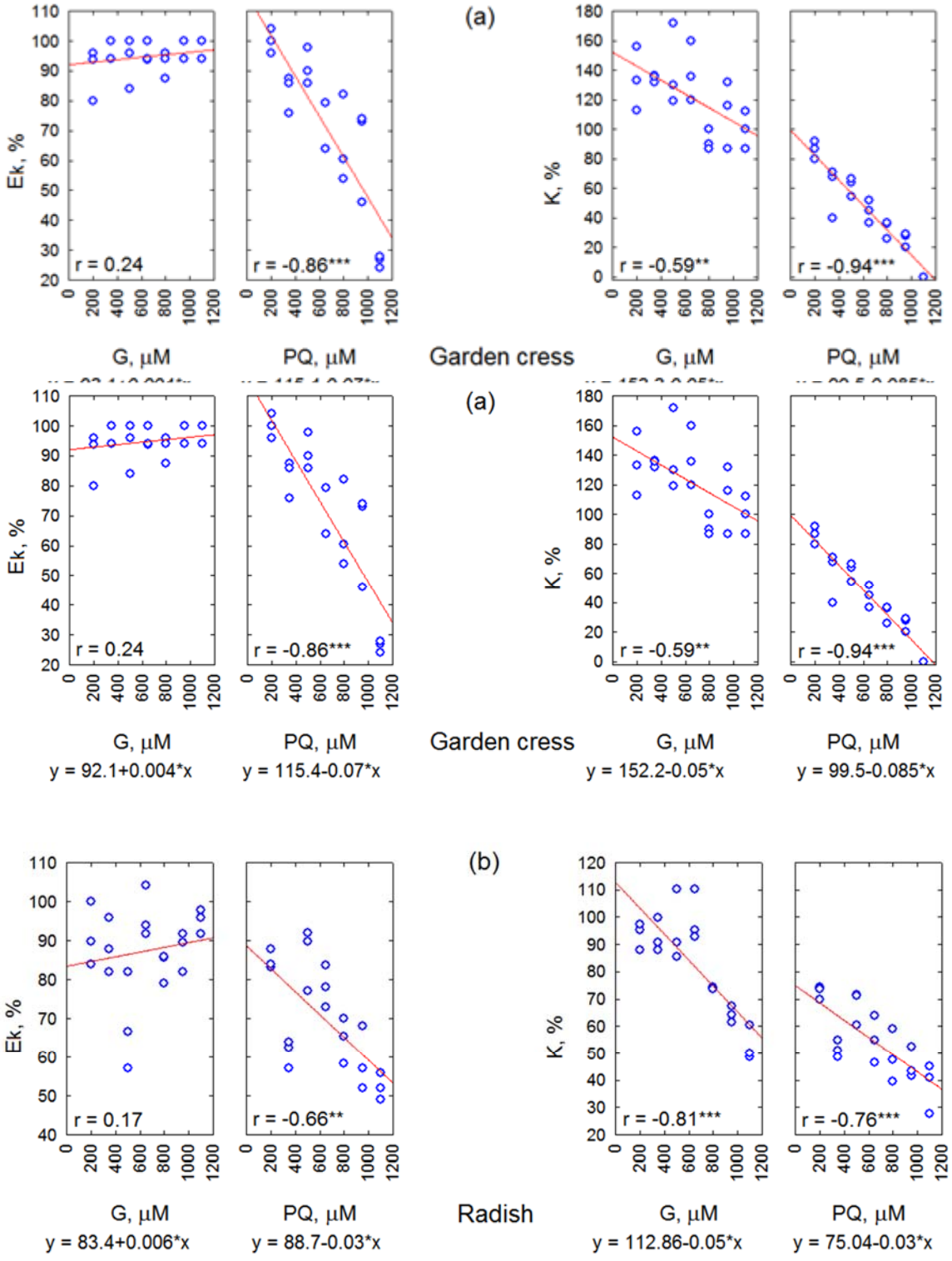

Figure 3. Scatterplots of correlations between Ek and K in % and the concentrations of the pesticides G and PQ for garden cress (a) and radish (b). Pearson's correlation coefficients (r) and regression equations were calculated (n = 21); ***p<0.001, **p<0.01, *p<0.05.

The glyphosate caused slight toxic effect on stems length up to 950 µM, where a sharp decrease was observed. The difference between the highest and the lowest percent in the roots length was 30 %. There was no clear trend during G treatment as the effect was localized at certain concentrations. The dependences between Lr, Ls and tested concentrations of G were polynomic functions of 2nd degree (Figure 4c and 4d).

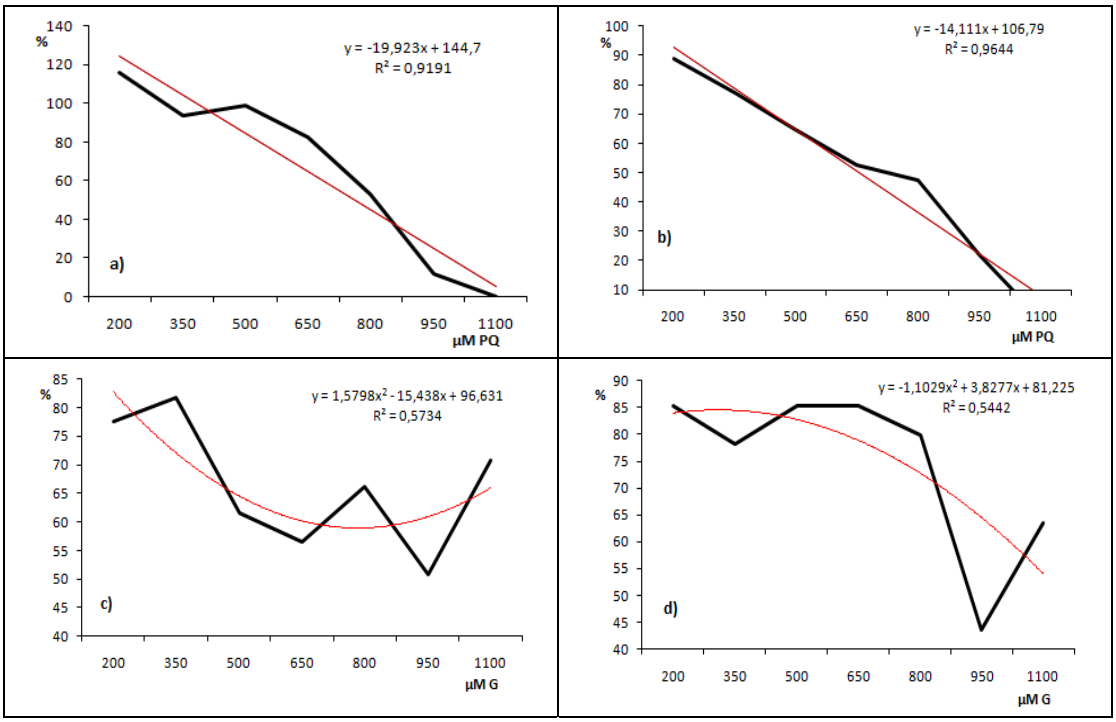

Figure 4. Ls and Lr of garden cress treated with different concentrations of PQ and G (% of the control): a) Lr with PQ treatment, b) Ls with PQ treatment, c) Lr with G treatment, d) Ls with G treatment.

3.2.2. Radish

The roots and stems of radish showed different sensitivity to the two pesticides. PQ caused higher toxic effect on root length (EC_{50}=177.48 µM) than G (EC_{50}=810.79 µM) (Figure 5a and 5c). On the other hand, PQ had stimulating effect on the stems at 500 µM (Figure 5b). At G treatment, the effect on roots and stems was definitely negative - their length decreased with increasing the pesticide concentration (Figure 5c and 5d). The dependences between Lr, Ls and tested concentrations of PQ and G were linear functions (Figure 5a, 5c and 5d). Only impact of PQ on the stems was described with polynomic functions of 2nd degree (Figure 5b).

The conducted experiments for PQ effect on the roots and leaves of the two test objects showed inhibition of their growth. The pesticide exerted higher effect on the garden cress compared to the radish and on the roots' growth compared to the leaves. The tests with G on roots and leaves showed also inhibition on their growth. In this case the radish was more sensitive test object than the garden cress.

The statistical analyses proved the toxic effect of two pesticides on the root and stem growth of tested plants (Figure 6a, 6b). Negative correlations of lengths with increasing pesticides concentrations was established. The correlations were strong to very strong of PQ influence on the garden cress and of G on the radish, which is related to the specificity of the tested plants. The dependence was also very strong of the PQ influence on the radish root growth. PQ had greater inhibitory effect on garden cress ($r \geq -0.90$) than G ($r < -0.60$) (Figure 6b). Results show also that PQ did not exert toxic effect only on radish stem that was also not statistically proven ($p > 0.05$).

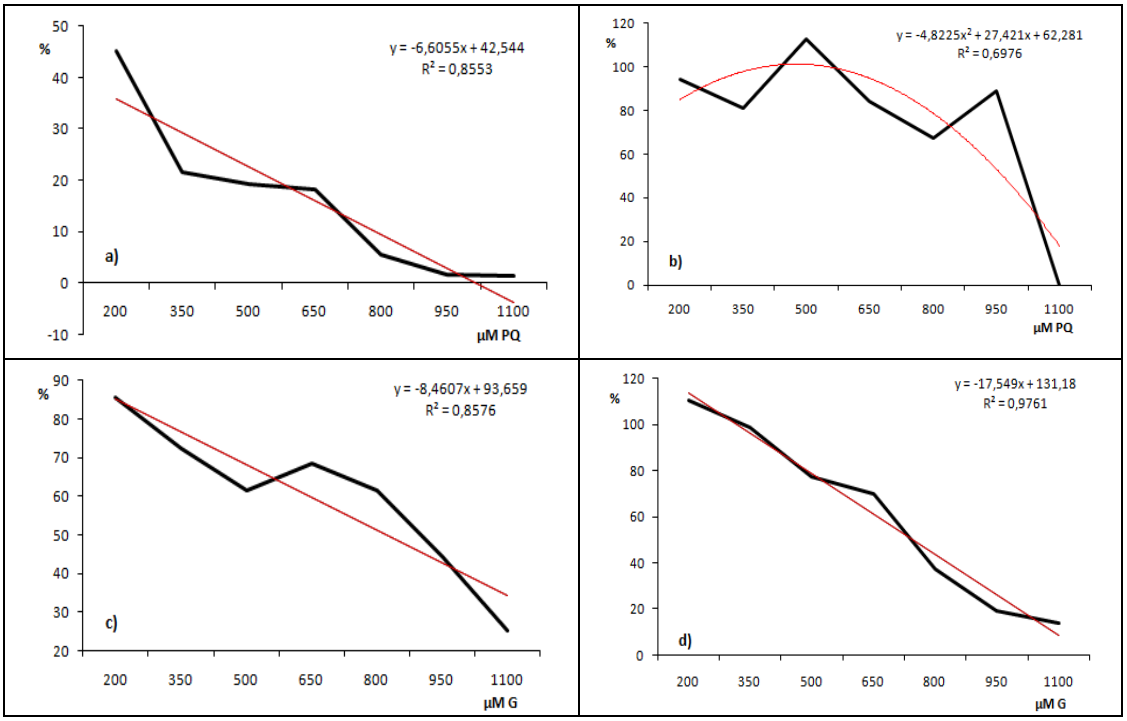

Figure 5. Ls and Lr of the radish with G and PQ treatment with different concentrations of PQ and G (% of the control): a) Lr with PQ treatment, b) Ls with PQ treatment, c) Lr with G treatment, d) Ls with G treatment.

Moore et al. (1999) also observed reduction in *T. latifolia* root and stem treated with paraquat. Some authors report even higher effect on the roots length. For example, Fatiha and Fouad [40] observed 75% reduction of *Lycopersicum esculentum* root length compared to control root. Similar to their results are those of Mitra and Raghu [42] who treated *A. hypogaea and B. juncea* seeds with DDT. The effect of pesticides on growth was reported in other studies as 50 % decrease of root growth was observed in *Phaseolus vulgaris* and *Pisum sativum* after treatment with chlorsulfuron during the germination process [43]. Fayez et al. [44] reported that *Zea mays* primary roots of the seedlings grew significantly slower in the presence of chlorsulfuron and metsulfuron methyl. This reduction could be explained by destruction of auxins due to the increase in the amount of phenols [45].

3.3. Influence of paraquat and glyphosate on the absolute dry weight of sprouts (% of the control)

3.3.1. Garden cress

The dry weight of stems treated with PQ was higher than the control at 200 µM while for G it was at 350 µM and 650 µM (EC_{50} = 854.68 µM) (Figure 7a). Very high stimulation on leaves was registered, caused by G as their dry weight reached its maximum at 350 µM (6.8 times higher than the control) (Figure 7b). Such high values were measured at all concentrations of the pesticide as they decrease drastically at 950 µM and 1100 µM to 80 and 20%, respectively. PQ had stronger inhibition and caused stimulation only at the lowest concentrations when the dry weight of the leaves reached 160% of that of the control. The roots were the most influenced from the pesticides (Figure 7c) as PQ exerted higher toxic effect reaching 100% lethality at 800 µM and 950 µM, while for G this effect was observed at the two highest concentrations. As a whole, the effect on the dry weight of the plant was localized at the different concentrations.

3.3.2. Radish

The highest values were recorded at 500 µM, 650 µM and 800 µM as for leaves there was also a stimulating effect at these concentrations reaching up to 2 times the dry weight of the control (Figure

8). The roots were most influenced from the toxic effect of the pesticides and EC_{50} for PQ was 338.91 µM (Figure 8c). PQ had higher toxic effect in all concentrations except the lowest and the highest (without the leaves). For both test objects G had stimulating effect on the leaves and PQ exerted more toxic effect as roots were the most sensitive. The weight increase was probably related to the biochemical features - the herbicides accumulation in biomass and/or the synthesis of stress hormones.

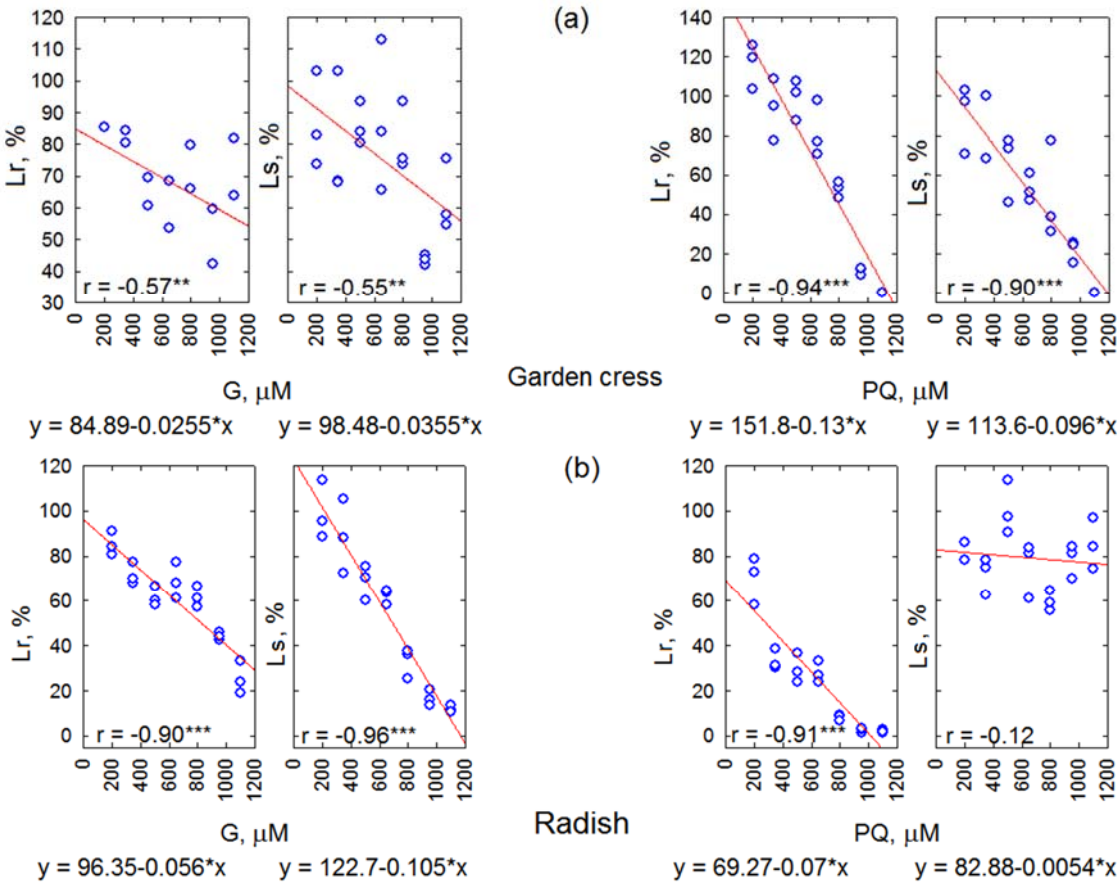

Figure 6. Scatterplots of correlations between Lr and Ls (in %) and the concentrations of the pesticides G and PQ for garden cress (a) and radish (b). Pearson's correlation coefficients (r) and regression equations were calculated (n = 21); ***p<0.001, **p<0.01, *p<0.05.

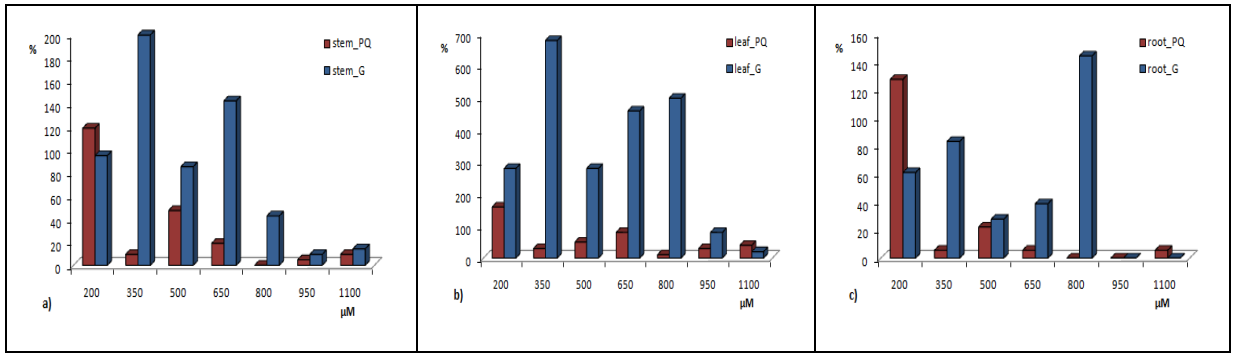

Figure 7. Absolute dry weight of stems (a), leaves (b) and roots (c) of garden cress (%) treated with PQ and G treatment.

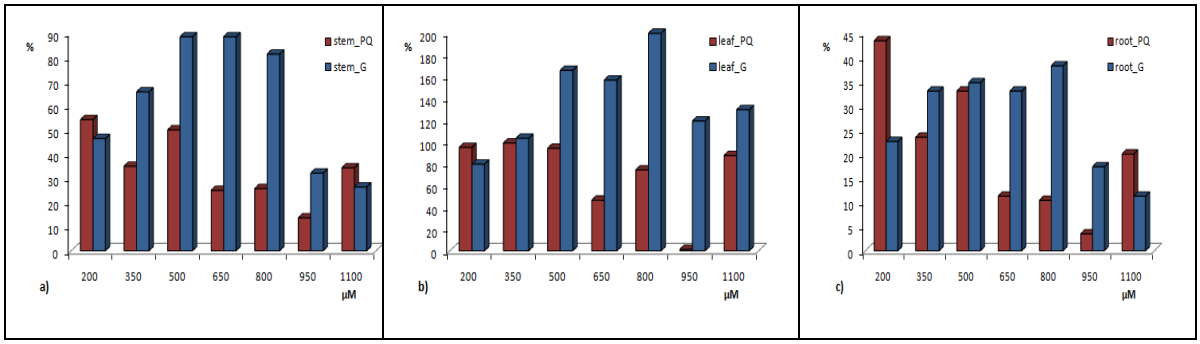

Figure 8. Absolute dry weight of stems (a), leaves (b) and roots (c) of radish (%) treated with PQ and G

The additional statistical data analyses showed that all obtained mean values were representative (Figure 9a, 9b). The differences between the mean values of the indicators at the different concentrations of two pesticides were significant, except Ek of the garden cress for G (Figure 9a). Only higher glyphosate concentrations affected the germination and Ls of the garden cress (Figure 9a) and their levels of significance were lower ($p < 0.05$) than all others ($0.01<p < 0.001$). Paraquat had more toxic effect than glyphosate on the garden cress (Figure 9a), but glyphosate - on radish Ls, and both herbicides - on radish Lr (Figure. 9b).

The comparison of indicators' means between the two herbicides (two-way ANOVA, Figure 10a, 10b) showed no significant differences between G and PQ for Lr of the garden cress (around 70%) and for Ls of the radish (55%), but significant differences ($p < 0.001$, except Ls of the garden cress with $p = 0.006$) for the others. Higher mean values were registered for G treatment for both plants. The strongest was impact of paraquat on K of the garden cress (from 120% for G, decreasing to 45% for PQ) and on Lr of the radish (from 60% to 25%). Wilk's lambda tests for all indicators of both plants (Figure 10a, 10b) showed $p<0.001$.

3.4. Genetic analysis

In this study, an optimization of temperature of annealing (Ta˚C) of each primer was firstly performed using 4 different Ta˚C: 45° C, 50° C, 55° C, and 60° C using radish DNA isolated from treated with 500 µM PQ and 950 µM G radish plants. As suitable temperatures of annealing were selected those producing clear and reproducible amplification products. Additional optimization of the Ta˚C (+/- 1-2° C) was also performed in order to obtain the clearest PCR profiles. Among the primers used in ISSR profiling only primers consisting of tri-nucleotide repeats did not yield any PCR products at the tested PCR conditions and hence these were eliminated from further experiments.

After optimization of PCR conditions DNA from each control and treated with 500 µM paraquat and 950 µM glyphosate radish plants, respectively were subjected to PCR using the selected PCR conditions (Table 1). The applied eight dinucleotide ISSR primers produced in a total 87 amplicons. Among them only ISSR3 and ISSR6 primers generated PCR amplicons differing between the herbicide-treated and the control samples. The identified polymorphisms in the ISSR profiles of the plants treated with different herbicides were distinct. A fragment of approximately 1400 bp was observed in the ISSR3 profile of G treated plants (950 µM) which was not observed in the profiles of both control and PQ treated plants (500 µM). However, in the profile generated with primer ISSR6 an allele of approximately 1300 bp was identified in both G and PQ treated plants in comparison to the control material (Figure 11).

The study showed mutation induced rearrangements in DNA of the herbicide treated plants. Probably medium to high herbicide dose treatments led to DNA variation such as single nucleotide mutations (SNPs), short insertion/deletions and/or activation of transposable elements in the genome of the treated plants. Such rearrangements are potential additional adaptive mechanisms for herbicide resistance of plants.

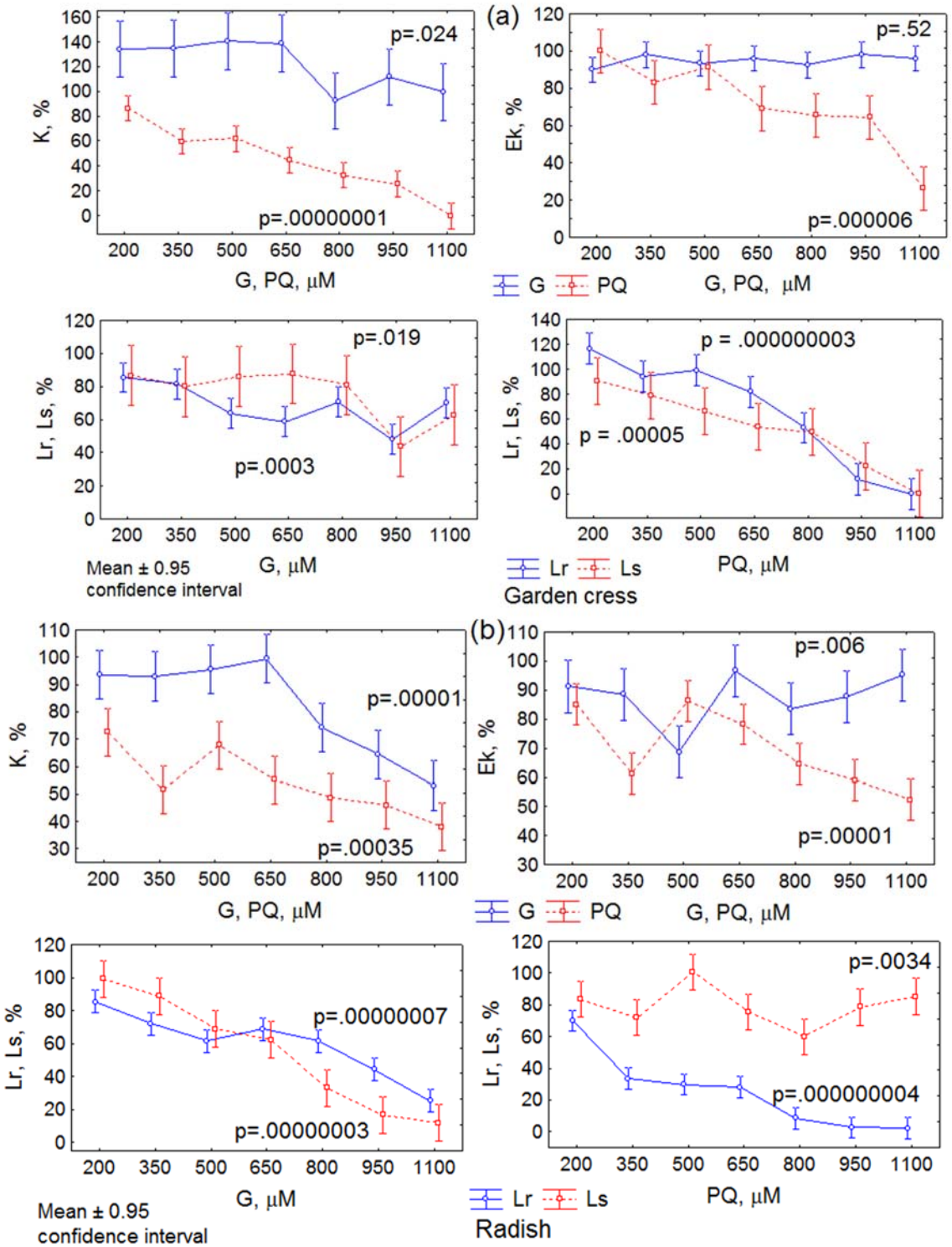

Figure 9. Mean plots (with 0.95 confidence intervals) of K, Ek, Lr, Ls in %, depending on the concentrations of pesticides G and PQ for garden cress (a) and radish (b) with given p-values of univariate ANOVA (f-tests).

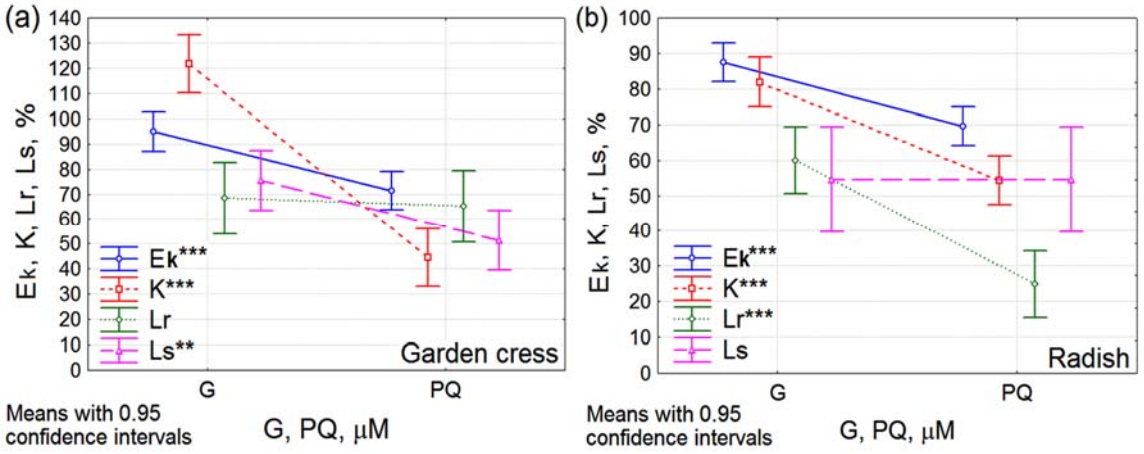

Figure 10. Mean plots (with 0.95 confidence intervals) of K, Ek, Lr, Ls in %, comparing two groups of pesticides G and PQ for garden cress (a) and radish (b) with mentioned p-values (***p<0.001, **p<0.01, *p<0.05; two-way ANOVA, f-test).

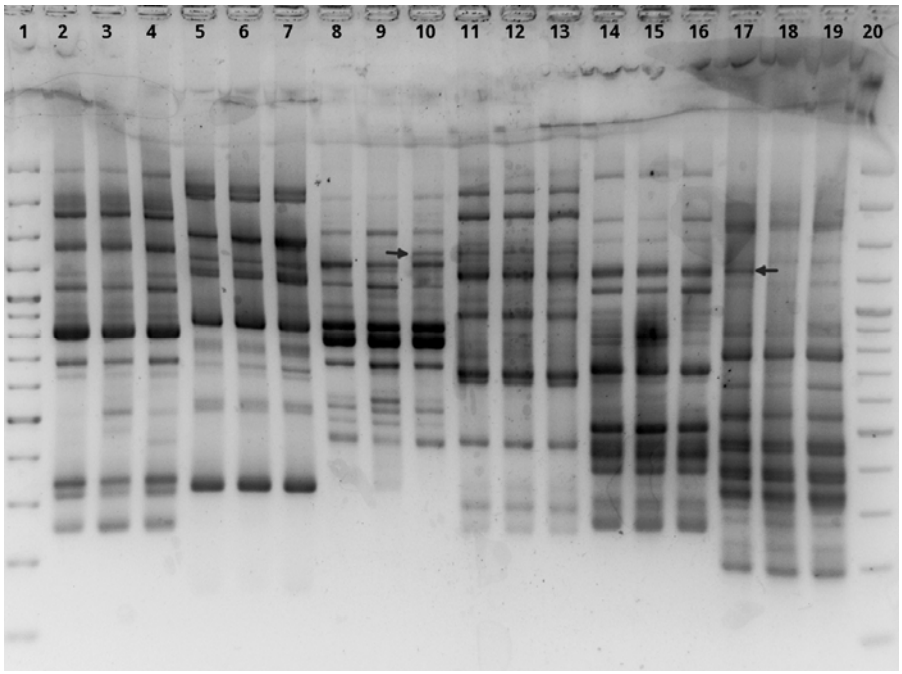

Figure 11. Agarose-gel electrophoresis of ISSR-PCR products: 1 - Marker (10bp ladder); 2-4 - ISSR1 profiles of Control, PQ and G treated plants respectively; 5-7 - ISSR2 profiles of Control, PQ and G treated plants respectively; 8-10 - ISSR3 profiles of Control, PQ and G treated plants; 11-13 - ISSR4 profiles of Control, PQ and G treated plants; 14-16 - ISSR5 profiles of Control, PQ and G treated plants; 17-19 - ISSR6 profiles of Control, PQ and G treated plants; 20 - Marker (100 bp Ladder). The arrows showed the polymorphic fragments.

ISSR profiling has been successfully performed to assess the genotoxic effect of heavy metals in *Eruca sativa* (L.) [46] and lead in *Pistia stratiotes* [47].

Recent studies [48, 49] showed that the abiotic stress induced by herbicide treatment can induce specific alterations of the plant methylome. The methylC sequencing of *Arabidopsis thaliana* leaves

developed after either mock treatment or two different sub-lethal doses of the herbicide glyphosate, showed that the herbicide injury resulted in 9205 differentially methylated regions (DMRs) across the genome [48]. Among them, more than a half DMRs were induced in a dose-dependent manner and the methylation levels were positively correlated to the severity of the herbicide injury. This fact suggests that plants can modulate the magnitude of methylation changes based on the severity of the stress. This study demonstrates that plants respond to herbicide stress through changes in DNA structure that are in general dose-dependent and, at least partially, stress-specific.

4. Conclusions

PQ has proven environmental toxicity and its usage is forbidden in Europe, while G is widely used in agricultural practice. Pollution of soil and surface water with pesticides can cause a reduction in biodiversity and species abundance, alteration in the structure of populations with consequent degradation of terrestrial communities. Besides their ability to bioaccumulate and biomagnify on trophic chains and thus remain in the biotope over a long time in increasing concentrations, they can inhibit seed germination and early development of young plants in ecosystems.

The tests with two herbicides - G and PQ - showed a generally pronounced higher toxicity of PQ for all tested indicators. Despite lower G toxicity, identical trend patterns were observed and EC50 could be detected at some of the variants. It was observed low G toxicity for Ek for both test objects and better toxicity for K, as more sensitive was the garden cress. PQ had a marked toxicity on the stems length that increased with the concentration increasing. There was a stimulating effect on the test objects weights with the concentrations increasing of both herbicides. Despite the stimulatory effect, the weights of PQ treated samples were generally lower than the control values, and those of G treated samples were usually higher than the control values. The various effective concentrations have been identified leading to a change in the weights representing 50% of the established weights in the control plants. Although two herbicides were of different usage status, the two primers reveal differences in the profiles between the treatments with both herbicides as the character of the polymorphisms is different. The experimental results showed that even G exerted less toxic effect on test objects; the trends in the investigated parameters were similar with those of the PQ. This is of great concern because its wide use can cause accumulation in soil and increasing of the effect on planted cultures.

This study demonstrates that plants respond to herbicide stress through changes at DNA level. The applied ISSR profiling technology is an appropriate tool for identification of both G and PQ induced mutations.

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