NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 37, 2020
Print ISSN 1222–4227; Online ISSN 2067–5720

PHYTOTOXIC AND CYTO-GENOTOXIC POTENTIAL OF *Phytolacca americana* ON *Zea mays*

Aurel Liviu Olaru, Dorina Bonea*, Elena Bonciu

University of Craiova, Faculty of Agronomy, Department of Agricultural and Forestry Technology,
19 Libertăţii str., 200421 Craiova, Dolj County, Romania
*Corresponding author. E-mail: dbonea88@gmail.com

ABSTRACT

Pokeweed (*Phytolacca americana* L.) is an invasive weed commonly found in the Oltenia area (Romania). Considering the economic importance of maize crop, this study was carried out to investigate the phytotoxic, cytotoxic and genotoxic potential of pokeweed extracts on maize (*Zea mays* L.). The seeds of maize were treated with different concentrations (5%, 10% and 20%) of pokeweed extracts, under laboratory conditions, for macroscopic and microscopic analyses, respectively. A control (distilled water) was used for comparison. All extracts significantly decreased germination, root and shoot length of maize seedling, also inhibited root cell division by the reduction of the mitotic index and by the induction of chromosomal and nuclear abnormalities in the root cells of maize. Chromosome stickiness, C-mitosis, piknosis, laggards, micronuclei and nuclear erosion, were observed. Thus, the present study suggests that the allelochemicals in the extracts of *P. americana* have a highly toxic potential on maize, so prevention and control of this invasive plant in the agricultural fields, are really necessary.

Keywords: allelopathy, invasive plant, maize, pokeweed.

INTRODUCTION

Invasive alien species are considered a threat against biodiversity all around the world. Once entered into a recipient ecosystem, an invasive plant can occupy large areas creating problems for the native flora and fauna. In cultivated areas, they can cause economic losses to farmers by reductions in crop yields and crop quality (Bhuler et al., 1998)

Several reports have shown that allelopathy plays a key role in invasion process, particularly when invaders release compounds that reduce germination, growth and/or reproduction of neighbouring plant species, as well as of the same species, in both natural and agricultural systems (Bich and Kato-Noguchi, 2014; Callaway and Aschehoug, 2000; Inderjit-Seastedt et al., 2008).

Allelopathy is a biological phenomenon by which one plant produces allelochemicals that inhibits the growth of other plants in the vicinity. The study of allelopathy increased in the 1970 and has undergone rapid development since the mid-1990, becoming a popular topic in ecology, agronomy, and other areas of inquiry in recent years (Cheng and Cheng, 2015).

Allelochemicals are liberated by different plants parts (roots, leaves, flowers, fruits) by leaching and volatilization, as well as from the soil through the decomposition of plant residue and root exudation (Pisula and Meiners, 2010; Rajendiran et al., 2014).

Phytolacca americana (Phytolaccaceae L. family) synonyms: *P. decandra*, *P. vulgaris*, common names: pokeweed, pocan, pokeberry, scoke, garget, pigeonberry. *P. americana* is a herbaceous perennial plant, native to the eastern part of North America. This invasive plant got into Europe in the 17th century. It was first cultivated in areas around the Mediterranean Sea, since 1650 and from 1770 it started to spread out from Bordeaux (France). Later it started to spread to the northern and eastern parts too (in the Mediterranean countries, the Benelux states, Germany, Switzerland, Austria, Slovakia, Hungary, Ukraine, southern part of Russia Croatia, Serbia-Montenegro, Romania and

Received 19 September 2019; accepted 17 November 2019. First Online: December, 2019. DII 2067-5720 RAR 2020-23

Bulgaria (Balogh and Juhász, 2008). The frugivorous bird species represent the primary vector for the diffusion of this species (Guzzetti et al., 2017).

Pokeweed is now emerging as an invasive weed in agricultural crops of Oltenia (Romania), because the local ecological conditions favour its growth (Niculescu and Cismaru, 2013). From agricultural point of view pokeweed is considered to be a weed in its native range, for example in maize, soybean and other crops with no tillage (Balogh and Juhász, 2008). It is known that, generally, the weeds affect the growth and development of maize when they coexist in the agricultural fields. Depending on the degree of infestation, weeds can reduce maize yield up to 90% (Dalley et al., 2006).

Maize (*Zea mays* L.) is an important cereal crop of the world, holding second position after rice in terms of production. It is used as food, fodder and a raw material in industries. In Romania, maize was produced on 2,405 thousand hectares with the production of 14,326 thousand tons and average yield of 5.9 tons ha^{-1}, in 2017 (FAO, 2017 http://www.fao.org).

Allelopathic potential of pokeweed on different crops such as *Cassia mimosoides, Brassica pekinensis, Plantago virginica, Digitaria sanguinalis* (Kim et al., 2005 a; Yang et al., 2012), has been previously reported.

Allelochemicals reported in *P. americana* are phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, m-hydroxybenzoic acid, coumaric acid, and cinnamic acid), triterpenoidal saponins, triterpene alcohol, lignanes, flavonoid setc (Kim et al., 2005 a, b; Ravikiran et al., 2011; Williams et al., 2002).

As far as we know, no information is available about the allelopathic effects of pokeweed on maize. Therefore, in the present study we investigated the allelopathic effects of pokeweed on germination and seedling growth of maize. Also, given a lack of references about cytotoxic and genotoxic potential of pokeweed, we investigated these effects to maize by cytological study.

MATERIAL AND METHODS

Plant material and preparation of the aqueous extracts

Experiment was conducted in 2018 in the Laboratory of Breeding plants at the Faculty of Agronomy in Craiova. The seeds of maize (‘P0937’ hybrid) were purchased from seed company DuPont Pioneer (Pioneer Hi-bred, Romania). Pokeweed plants (*P. americana*) were collected in November, at their mature stage (with fruits), from the areas surrounding cultivated fields (Oltenia area). The aboveground parts were dried at room temperature (25-30°C) and ground with electronic grinder into powder.

The aqueous extracts were prepared according to modified method of Norsworthy (2003), from fresh and dry material, by mixing 200 g of plant powder with 1000 ml of distilled water. This mixture was kept for 24 h at room temperature and filtered through filter paper. The obtained extracts were diluted with distilled water to obtain three final concentrations of 5%, 10% and 20%. The effects of the extracts were compared with the control (distilled water, considered 0%).

The final seed germination was calculated using the formula:

$$G\ (\%) = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

After seven days, root and shoot length were measured.

Treatments of the maize seeds with aqueous extracts

Maize seeds were disinfected with 1% NaOCl (4% NaOCl commercial bleach), for 20 min., then rinsed three times with distilled water (Siddiqui et al., 2009). Twenty-five seeds were placed in sterilized plastic casseroles on top of filter paper. In each casserole we added 15 ml of aqueous extract, while distilled water was used in control. The plastic casseroles were placed in the grow the chamber (Binder KBF 720, Binder manufacturer, USA) at 25°C, 80% humidity and in the dark, for 7 days. All treatments had four replications.

Cytogenetic analysis

The maize root tips were fixed in a mixture of glacial acetic acid (GAA) and ethanol in a volume ratio of 1:3, for 12 hours at room temperature.

For chromosomal analysis, the root tips were hydrolysed in1 mol/L HCl for 5 min. and transferred for colouring stage in a basic fuchsine solution, in concentration of 10%. The tip (2-3 mm) of the root was then cut and placed on a glass slide in a drop of acetocarmine solution. The microscopic slides were prepared using the squash technique, a common procedure for chromosomes study in plant cytogenetic. Five slides for each variant were analysed for calculating the mitotic index and the total abnormalities frequency, comprising both chromosomal aberrations and nuclear anomalies. All slides were examined using an optical Kruss microscope with digital camera (Kruss manufacturer Hamburg, Germany).

The Mitotic Index (MI) was calculated as the number of dividing cells per number of observed cells (Fiskesjo, 1997), using the following formula:

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

In this study we evaluated mitotic aberrations (stickiness, laggards, C-Mitosis) and nuclear anomalies (pyknosis, micronuclei and cells with nuclear erosion).

The index of the Total abnormalities (TA) was calculated using the following formula:

$$TA\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

Statistical analyses

The obtained data were analysed statistically with one-way analysis of variance (ANOVA) using MS Excel 2007. The differences between treatment means were compared using the LSD-test at probability level of 0.05 (Săulescu and Săulescu, 1967).

RESULTS AND DISCUSSION

Effect of aqueous extracts on seed germination and seedling growth

The *P. americana* aqueous extract exerted a significantly phytotoxic effect on the germination of the *Z. mays* seeds ($p \leq 0.05$). For the control, the germination was 88%. The degree of inhibition of the germination increased with the increase of the concentrations of extracts, but we can say that all the used concentrations led to a drastic inhibition of the maize germination.

The highest concentration of 20% resulted in 73.8% (23 cm) reduction in germination over the control (Table 1). The extracts of *P. americana* at all concentrations inhibited the root length and shoot length of *Z. mays* compared with the control, and the degree of inhibition increased with increasing aqueous extracts concentration. At the control, the root length and shoot length was 12.35 cm and 6.32 cm, respectively. The highest concentration of 20% determined reduction of the root length and shoot length with 69% (3.82 cm) and 78.3% (1.37 cm) compared to the control value (Table 1).

Table 1. Effect of *P. americana* aqueous extracts on germination and seedling growth of maize

P. americana extracts (%)	Germination (%) (± SE)	Root length (cm) (± SE)	Shoot length (cm) (± SE)
0 (Control)	88 ± 4.90	12.35 ± 0.56	6.32 ± 0.23
5	35 ± 1.91*	6.1 ± 0.32*	3.50 ± 0.26*
10	28 ± 1.63*	4.8 ± 0.07*	1.95 ± 0.13*
20	23 ± 1.91*	3.82 ± 0.25*	1.37 ± 0.19*

Note: * - significant at probability level ≤0.05; SE - Standard error.

Effects of extracts of *P. americana* on mitotic index and chromosomal aberration assay

Cytogenetic analysis revealed that, in comparison with control, exposure of *Z. mays* to extracts of *P. americana* at all concentrations produced significant ($p \leq 0.05$) reductions of the mitotic index (MI) in the root meristematic cells. Mitotic index showed a steady decrease with increasing concentrations of all the extracts. The highest value of the mitotic index (19.21%) was recorded at the 5% concentration, and the lowest value of the mitotic index (9.64%) was observed at the 20% concentration (Table 2).

Table 2. Effect of pokeweed extracts on mitotic index (MI) of maize

P. americana extracts (%)	Total no. of observed cells	Total no. of dividing cells	MI (%) (± SE)
0 (Control)	1134	356	31.39 ± 0.52
5	1176	226	19.21 ± 1.02*
10	1249	183	14.65 ± 0.47*
20	1254	121	9.64 ± 0.93*

Note: * - significant at probability level ≤0.05; SE - Standard error.

Application of extracts of the pokeweed changed the normal cycle of events of mitosis in *Z. mays* root tip cells and induced the appearance of some chromosomal abnormalities. The highest value of TA (%) was recorded at concentration of 20% (19.83%). The most common chromosomal aberrations were piknosis, C-mitosis and stickiness. Other types of aberrations such as laggards, micronuclei, nuclear erosion were also observed [Table 3 and Figure 1 (a-f)].

Table 3. Frequency of chromosomal aberration in total number of observed cells induced by pokeweed extracts in root tip cells of maize

P. americana extracts (%)	Total no. of dividing cells	Total no. of aberrant cells	TA (%)	Chromosomal aberration and nuclear anomalies (%)					
				S	CM	L	PK	MN	NE
0 (Control)	356	10	2.8	5(50.0)	3(30.0)	2(20.0)	0	0	0
5	226	26	11.50	4(15.4)	6(23.1)	5(19.2)	6(23.1)	2(7.7)	3(11.5)
10	183	29	15.84	6(20.7)	7(24.1)	1(3.4)	10(34.5)	2(6.9)	3(10.3)
20	121	24	19.83	4(16.7)	6(25.0)	1(4.16)	9(37.5)	2(8.3)	2(8.3)

Note: TA = Total abnormalities index (%); S = Stickiness; CM = C- mitosis; L = Laggards; PK = Piknosis; MN = Micronuclei; NE = nuclear erosion.

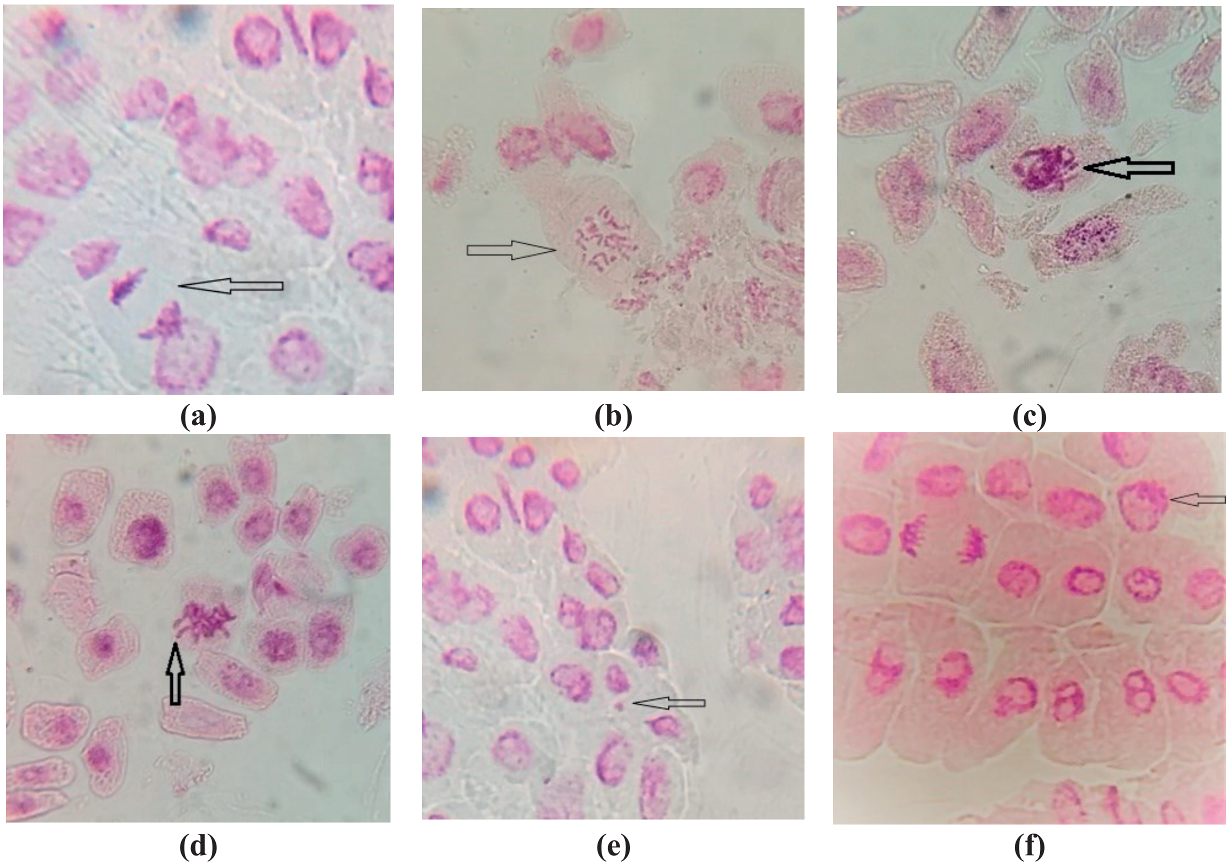

Figure 1. Some chromosomal aberrations and nuclear anomalies induced by the extract of *P. americana* to *Zea mays*: sticky chromosomes in anatelophase (a); C-mitosis (b); pyknosis (c); laggards (d); micronucleus (e); cell with nuclear erosion (f).

Allelopathy is a natural ecological mechanism. Many invasive species produce large amounts of allelochemicals, thus increasing their ability to invade plants communities (Inderjit-Seastedt et al., 2008; Kim et al., 2005 a). This strategy is known as a mechanism or a theory of "novel weapons hypothesis" (Callaway and Ridenour, 2004)

The allelopathic experimental system under laboratory conditions is commonly used (simple, rapid) to study the toxic effects of some noxious chemicals (Morsi and Abdelmigid, 2016).

In this study, phytotoxic effects of pokeweed extracts were evaluated by analysing the seeds germination and seedlings growth of maize. All aqueous extracts significantly inhibited seeds germination, root and shoot length of maize. This may be due to some allelochemicals present in the extracts of pokeweed which caused the death of embryos or prevented growth of seedlings. According to Ferrarese et al. (2000), the phenolic compounds are allelochemicals that induce the increase of the oxidative enzymes activity, having as consequence the cell wall lignification, reducing the root growth.

A large number of chemicals were found to be present in different organs of the *P. americana* plant. The presence of phenolic compounds in the leaf, in the berries and in the seeds, throws a light on its inhibitory activity (Kim et al, 2005 b; Nabavi et al., 2009; Woo and Kang, 1979).

The phytotoxic effect of some species in the Phytolaccaceae family on the growth and development of some plant species, including on agricultural species (but not on maize), has already been reported in many scientific papers. For example, the *Phytolacca americana* extracts drastically inhibited the germination of *Lactuca indica* and *Sonchusoleraceus* seeds (Kim et al., 2005 b), seed germination and seedling growth of *Brassica pekinensis, Plantago virginica* and

Digitaria sanguinalis (Yang et al., 2012). Ullah et al. (2015) reported that *Phytolacca latbenia* extracts inhibited *Triticum aestivum* and *Brassica napus* seed germination and seedling growth.

Our study confirmed that *P. americana* had a strong phytotoxic potential, inhibiting the germination and growth of maize seedlings.

Cyto-genotoxicity was estimated by observing some cytological parameters, such as the mitotic index and presence of chromosomal abnormalities. In this study, we observed that all concentrations of *P. americana* aqueous extracts determined a significant reduction of the mitotic index compared to the control.

Very few studies are available for cyto-genotoxicity of invasive species on maize, and for *P. americana* cyto-genotoxicity we have not found bibliographic references. Abderrahman (1998), and Bonea et al. (2018), showed that the *Peganum harmala* extracts and *Ambrosia artemisifolia,* respectively, reduced the mitotic index value to *Zea mays* and induced the occurrence of some abnormalities, such as: multinucleated cells, C-metaphases and sticky, ring, fragment and bridge-type chromosomes.

According to Amin (2002), the cyto-genotoxic level of some components/chemicals in a plant or environmental extract can be determined based on the increase or decrease of the mitotic index in the cells exposed to the tested chemicals, which constitute an appropriate test in bio-monitoring studies. To investigate the genotoxic potential, chromosome aberrations and frequency of cells with micronuclei have been considered an efficient indicator of the cytogenetic effects (Bonciu et al., 2018; Carita and Marin-Morales, 2008; Krishna and Hayashi, 2000).

In our study, a higher percentage of abnormalities (TA) in meristematic roots of maize were observed for the 20% and 10% concentration. From this point of view, pyknosis was the type of the chromosomal aberration that recorded the highest frequency at these concentrations. Similar results were reported by Caetano-Pereira (1995), who noticed that the pyknosis occurred at considerable frequency in maize cells under aluminium influence. In severe cases of stickiness, the lack of chromosome separation determined the formation of single or multiple pyknotic nuclei which culminated in full chromatin degeneration. According to Mendez-Veira et al. (2005), pyknosis (pyknotic nuclei placed in the cell centre) is an abnormality generally caused by chromosome stickiness. The presence of C-mitosis aberrations is commonly associated with spindle poisoning (Shahin and El-Amoodi, 1991), the presence of stickiness reflecting highly toxic and usually irreversible effect that probably leads to cell death (El-Ghamery et al., 2000) and lagging of chromosomes is due to abnormal spindle activity (Lera and Burkard, 2012). Micronuclei can be a result of acentric fragments, laggard chromosomes or even a malfunctioning spindle (Sudhakar et al., 2001).

Our study showed that the aqueous extracts from the aerial part of the *P. americana* species induced a strong mito-depressive effect, inhibiting the early growth of the maize seedlings.

CONCLUSIONS

The results of the present study indicated that all the tested extracts significantly decreased the germination, the root length and shoot length of maize seedlings. The extracts proved to be highly effective in eroding the chromosomes of maize, because they produced significant reductions in the mitotic index and induced chromosomal and nuclear abnormalities in the root cells of maize. So, we observed the presence of chromosome stickiness, C-mitosis, piknosis, laggards, micronuclei and nuclear erosion.

Thus, the present study showed and cautions that the pokeweed has a strong phytotoxic and cyto-genotoxic potential, and for this reason it is opportune to prevent and control this invasive plant in the agricultural fields.

REFERENCES

Abderrahman, S.M., 1998. *Cytogenetic effects of Peganum harmala extract on maize root tips*. Cytologia, 63(3): 283-291.

Amin, A.W., 2002. *Cytotoxicity testing of sewage water treatment using Allium cepa chromosome aberration assay*. Pakistan Journal of Biological Science, 5(2): 184-188.

Balogh, L., Juhász, M., 2008. *American and Chinese pokeweed* (*Phytolacca americana, Phytolacca esculenta*). In: The most important invasive plants in Hungary, by Botta-Dukát Z., Balogh L., CER Institute of Ecology and Botany, Vácrátót, Hungary: 35-46.

Bich, T.T.N., Kato-Noguchi, H., 2014. *Isolation and identification of a phytotoxic substance from the emergent macrophyte Centrostachys aquatica*. Botanical Studies, 55: 1. DOI: 10.1186/s40529-014-0059-1

Bonciu, E., Firbas, P., Fontanetti, C., Wusheng, J., Karaismailoğlu, M.C., Liu, D., Menicucci, F., Pesnya, D., Popescu, A., Romanovsky, A., Schiff, S., Ślusarczyk, J., Souza, C., Srivastava, A., Sutan, A., Papini, A., 2018. *An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay*. Caryologia, 71(3): 191-209.

Bonea, D., Bonciu, E., Niculescu, M., Olaru, A.L., 2018. *The allelopathic, cytotoxic and genotoxic effect of Ambrosia artemisiifolia on the germination and root meristems of Zea mays*. Caryologia, 71(1): 24-28.

Bhuler, D.D., Netzer, D.A., Riemenschneider, D.E., Hartzler, R.G., 1998. *Weed management in short rotation poplar and herbaceous perennial crops grown for biofuel production*. Biomass Bioenergy, 14(4): 385-394.

Caetano-Pereira, M.A., Pagliarini, M.S., Brasil, E.M., Martins, E.N., 1995. *Influence of aluminum in causing chromosome stickiness in maize microsporocytes*. Maydica, 40: 325-330.

Callaway, R.M., Aschehoug, E.T., 2000. *Invasive plants versus their new and old neighbors: a mechanism for exotic invasion*. Science, 290(5491): 521-523.

Callaway, R.M., Ridenour, W., 2004. *Novel weapons: invasive success and the evolution of increased competitive ability*. Frontiers in Ecology and the Environment, 2: 436-443.

Carita, R., Marin-Morales, M.A., 2008. *Induction of chromosome aberrations in the Allium cepa test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes*. Chemosphere, 72(5): 722-725.

Cheng, F., Cheng, Z., 2015. *Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy.* Frontiers in Plant Sciences, 6: 1020. DOI: 10.3389/fpls.2015.01020

Dalley, C.D., Bernards, M.L., Kells, J.J., 2006. *Effect of weed removal timing and spacing on soil moisture in corn* (*Zea mays*). Weed Technology, 20(2): 399-409.

El-Ghamery, A.A., El-Nahas, A.I., Mansour, M.M., 2000. *The action of Atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of Allium cepa and Vicia faba*. Cytology, 65: 277-287.

FAO, 2017. *Statistical data base*. Food and Agriculture Organizations of the United Nations. Rome, Italia. http://www.fao.org/faostat/en/#data/QC

Ferrarese, M.L.L., Souza, N.E., Ferrarese-Filho, M.L.L., 2000. *Ferulic acid uptake by soybean root in nutrient culture*. Acta Physiologiae Plantarum, 22(2): 121-124.

Fiskesjo, G., 1997. *Allium test for screening chemicals; evaluation of cytological parameters*. In: Wuncheng W., Gorsuch J.W., Hughes J.S. (Eds.), Plants for Environmental Studies: 308-333. Boca Raton, New York: CRC Lewis Publishers. http://dx.doi.org/10.1201/9781420048711.ch11

Guzzetti, L., Galimberti, A., Bruni, I., Magoni, C., Ferri, M., Tassoni, A., Sangivvani, E., DellAgli, M., Labra, M., 2017. *Bioprospecting on invasive plant species to prevent seed dispersal*. Scientific Reports, 7(1): 1-11.

Inderjit-Seastedt, T.R., Callaway, R.M., Pollock, J.L., Kaur, J., 2008. *Allelopathy and plant invasions: traditional, congeneric and bio-geographical approach*. Biological Invasions, 10(6): 875-890.

Kim, Y.O., Johnson, J.D., Lee, E.J., 2005 a. *Phytotoxicity of Phytolacca americana leaf extracts on the growth and physiological response of Cassia mimosoides*. Journal of Chemical Ecology, 31(12): 2963-2974.

Kim, Y.O., Johnson, J.D., Lee, E.J., 2005 b. *Phytotoxic effects and chemical analysis of leaf extracts from three Phytolaccaceae species in South Korea*. Journal of Chemical Ecology, 31(5): 1175-1186.

Krishna, G., Hayashi, M., 2000. *In vivo rodent micronucleus assay: protocol, conduct and data interpretation*. Mutation Research, 455(1-2): 155-166.

Lera, R.F., Burkard, M.E., 2012. *High mitotic activity of polo-like kinase 1 is required for chromosome segregation and genomic integrity in human epithelial cells*. Journal of Biological Chemistry, 287(51): 42812-42825.

Mendes-Vieira, D., Mendes-Bonato, A.B., Pagliarini, M.S., Do Valle, C.B., 2005. *Abnormal meiotic behavior in Brachiaria brizantha (Poaceae) leading to microspore degeneration*. Caryologia, 58(4): 396-402.

Morsi, M.M., Abdelmigid, H.M., 2016. *Allelopathic activity of Eucalyptus globulus leaf aqueous extract on Hordeum vulgare growth and cytogenetic behavior*. Australian Journal Crop Science, 10(11): 1551-1556.

Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Bahramian, F., 2009. *In vitro antioxidant activity of Phytolacca americana berries*. Pharmacologyonline, 1: 81-88.

Niculescu, M., Cismaru, P.I., 2013. *Invasive plants from agricultural crops in Oltenia area*. Analele INCDA Fundulea, LXXXI: 103-112.

Norsworthy, J.K., 2003. *Allelopathic potential of wild radish* (*Raphanus raphanistrum*). Weed Technology, 17(2): 307-313.

Pisula, N.L., Meiners, S.J., 2010. *Relative allelopathic potential of invasive plant species in young disturbed woodland*. Journal of Torrey Botanical Society, 137(1): 81-87.

Rajendiran, K., Soumiya, D., Sudaroli Sudha, J., 2014. *Allelopathy and cytotoxicity of aqueous extracts of Lantana camara L. on Zea mays L. Var. Cauvery-244*. International Journal of Food, Agriculture and Veterinary Sciences, 4(1): 165-171.

Ravikiran, G., Raju, A.B., Venugopa, Y.L., 2011. *Phytolacca americana*: *a review*. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3(2): 942-946.

Săulescu, N.A., Săulescu, N.N., 1967. *Câmpul de experienţă*. Editura Agro-Silvică, Bucureşti. (In Romanian)

Siddiqui, S., Bhardwaj, S., Khan, S.S., Meghvanshi, M.K., 2009. *Allelopathic effect of different concentration of water extract of Prosopsis juliflora leaf on seed germination and radicle length of wheat (Triticum aestivum Var-Lok-1).* American-Eurasian Journal of Scientific Research, 4(2): 81-84.

Shahin, S.A, El-Amoodi, K.H., 1991. *Induction of numerical chromosomal aberrations DNA synthesis using the fungicides nimrod and rubigan-4S Vicia faba L*. Mutation Research, 261(3): 169-176.

Sudhakar, R., Ninge-Gowda, K.N., Venu, G., 2001. *Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa*. Cytologia, 66(3): 235-239.

Ullah, N., Haq, I.U., Mirza, B., 2015. *Phytotoxicity evaluation and phytochemical analysis of three medicinally important plants from Pakistan*. Toxicolology and Industrial Health, 31(5): 389-395.

Williams, L.A.D., Rosner, H., Conrad, J., Moller, W., Beifuss, U., Chiba, K., Nkurunziza, J.P., Kraus, W., 2002. *Selected secondary metabolites from the Phytolaccaceae and their biological/pharmaceutical significance*. Recent Research Developments Phytochemistry, 6: 13-68.

Woo, W.S., Kang, S.S., 1979. *A new phenolic aldehyde from the seeds of Phytolacca americana*. Korean Journal of Pharmacognosy, 10(2): 83-84.

Yang, S.H., Ziang, B.L., Zhou, B., Wang, N., Yang, J.Q., 2012. *Allelopathic activity of the extract from Phytolacca americana - an invazive alien plant*. Journal of Ecology and Rural Environment, 28(2): 139-145.