Journal of Animal & Plant Sciences, 31(6): 2021, Page: 1720-1727
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2021.6.0374

FOLIAGE APLLIED GIBBERELIC ACID INFLUENCES GROWTH, NUTRIENT CONTENTS AND QUALITY OF LETTUCES (*LACTUCA SATIVA* L.)

V. N. Le[1, 2], Q. T. Nguyen[1, *], N. T. Nguyen[1], T. Janda[3], G. Szalai[3], T. D. Nguyen[1] and T. G. Le[1]

[1]Center for Research and Technology Transfer (CRETECH), Vietnam Academy of Sciences and Technology (VAST), Hanoi 10072, Vietnam; [2]Graduate University of Sciences and Technology, VAST, Hanoi 10072, Vietnam; [3]Centre for Agricultural Research, Martonvásár 2462, Hungary
[*]Corresponding author's Email: nqt79@yahoo.com.

ABSTRACT

Plant growth regulators (PGRs) have been considered as agrochemicals applied widely in agricultural production, wherein, gibberelic acid (GA_3) has gained an interest in using to enhance the growth and improve yield for various vegetables. In this work, lettuces were sprayed with GA_3 at different concentration of 0, 10, 30, 50 and 100 mg L^{-1}, respectively, at three dates after transplanting (the 7-day, the 14-day, and the 21-day). The impacts of GA_3 on the growth, nutrient elements, and the absorption of heavy metals in lettuces were investigated. The accumulation of GA_3 by lettuce plants were confirmed by liquid chromatography - tandem mass spectrometer system (LC-ESI-MS/MS). The results demonstrated, that low concentrations of GA_3 (10 and 30 mg L^{-1}) increased the plant height, number of leaves and fresh weight of lettuces, whereas the opposite results were reported in high concentration of GA_3 treatment (100 mg L^{-1}). In addition, GA_3 decreased nutrient contents of Fe and Mn, and heavy metals' absorption in lettuces. However, the higher concentrations of GA_3 treatment use, the more their accumulations were found in lettuce samples. To the best of our knowledge, this is the first investigation of the influences of GA_3 on nutrient elements and the absorption of heavy metals in lettuce. This is needed to further investigate the improvement of crop production efficiency with PGRs, and to minimize their harmful impact on human health and the ecological environment.

Keywords: GA_3, heavy metals, lettuce, nutrients

Published first online March 31, 2021 Published final Nov. 20, 2021.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an economic value vegetable, which contains high content of vitamin A and several crucial minerals for the health of human namely calcium (Ca), iron (Fe), phosphorus (P), zinc (Zn), magnesium (Mg), manganese (Mn), and potassium (K) (Kim *et al*., 2016). It is widely used in salad mixtures with other vegetables like carrot, cucumber, and tomato… or in sandwiches. Various properties of lettuces were reported to improve health benefits in the previous studies. Fernanze *et al*. (1997) and USDA (2004) indicated that β-carotene and lutein in lettuces contributing to reduce colorectal cancer, cataracts, heart disease, and stroke in human; whereas phenolic compounds and carotenoids were responsible for antioxidant scavenging and possess antioxidant capacity properties, respectively (López *et al*. 2014). In addition, α-linolenic and omega-3 polyunsaturated fatty acids were found as the major fatty acid in lettuces (Pereira *et al*., 2001; Le *et al*., 2008).

Among the growth of population in the world, there is an increase in food consumption, especially vegetables, which might reduce the risks of cancer and degenerative diseases (Lynch *et al*., 2009; Soerjomataram *et al*., 2010; Khan *et al*., 2017). To meet consumers' increasing demands for green vegetables, it is critical to raise the productivity in vegetables with easy to use, cost-effective, and environmentally friendly techniques (Alessandro *et al*., 2019). There are many ways to reach these goals, for instance, genetic improvement, grafting, novel production systems, PGRs, and development promoting microorganism (Shah *et al*., 2006; Maggio *et al*., 2010; Khan and Samiullah, 2003).

PGRs have been considered as a new generation of agrochemicals applied in agricultural production and in horticulture to control the development processes of plant crops from germination to post-harvest preservation (Sunil *et al*., 2015). Among these, gibberellic acid (GA_3) has gained an interest in using to enhance the growth and improve yield for various vegetables, such as bean (*Phaseolus vulgaris*), rapeseed-mustard, black cumin (*Nigella sativa* L.), tomato, etc. (Emongor, 2002; Khan and Samiullah, 2003; Shah *et al*., 2006; Khan *et al*., 2006). In addition, GA_3 has been also applied to increase the tolerance of vegetables to abiotic stresses, including drought, salinity, salt, etc. (Shah, 2007; Maggio *et al*., 2010). Besides the effectiveness of GA_3 application in vegetable production, their influence on the nutrient contents, quality and safety of vegetable products to consumers need to be further studied.

In the present research, the influences of gibberellic acid on the growth, nutrient contents and quality of Lettuces (*Lactuca sativa* L.) were investigated. In addition, the absorption of heavy metals associated to GA_3 application was also studied, which might be the basic in exploring solutions for treatments of heavy metals in plant tissues.

MATERIALS AND METHODS

Chemicals: GA_3 was bought from East Asia Agricultural Limited company (Ho Chi Minh City, Vietnam) while certified standard of GA_3 (≥ 99%) and nutrient elements were bought from OlChemim Ltd. (Czech Republic). HPLC grade Methanol (MeOH; 99.80%) and formic acid (FA; ≥ 98%) were obtained from Sigma−Aldrich (Singapore). Deionized (DI) water was produced by using a Millipore Milli-q Integral 3 Water Purification System (France).

Instrumentation: The inductive coupled plasma-mass spectrometry (Agilent 7900 ICP-MS, Santa) was used to analyze the contents of heavy metals and nutrients in soil and plant samples. The optimized operating conditions of ICP-MS are Nebulizer gas flow rates: 0.88 L/min; Auxiliary gas flow: 1.2 L/min; Plasma gas flow: 16 L/min; ICP RF power: 1200 W; Lens voltage: 7.25 V; Analyzer vacuum: 5.0×10^{-7}. In addition, the instrument was located in a temperature-control laboratory (20±0.5°C), which ensure to stabilize for the optimization and analysis procedures.

Experimental design: Lettuce (*Lactuca sativa* L.) seeds were purchased from the Field Crops Research Institute (FCRI), Vietnam. Experimental lettuce seeds were sowed in moist soil for germination. After 7 days, similar seedlings of lettuces were selected and transferred to the pots (60 cm × 40 cm × 45 cm, length × width × height) containing fertilizers, with nine plants per box. Lettuces were sprayed by GA_3 at different concentration of 0, 10, 30, 50, and 100 mg L^{-1}, respectively, at three dates after transplanting (the 7-day, the 14-day, and the 21-day). GA_3 was dissolved in tap-water. All experiments were repeated in three replications and carried out from February to March, 2019 in the greenhouse of Vietnam Academy of Science and Technology with the temperature of 25 ±0.5 °C; and the air humidity of 70±0.5 %.

Determination of metal concentrations in the experimental soil: The soil samples (1.5 g) were prepared in a well-labelled 100 mL polytetraflouroethylene (PTFE) Teflon bombs and diluted with 6 mL (98%) HNO_3, 3 mL (35%) HCl and 0.25 mL (30%) H_2O_2 solutions. The digestion procedure was done in 30 min using the milestone ETHOS 900 microwave labstation (INSTR: MLS-1200 MEGA) (Tiimub and Darley, 2015; Adama *et al*., 2016). The samples were then centrifuged at 4000 rpm for 10 min, then filtered through 0.22 µm pore size PTFE membranes and diluted with deionized water before injection into the ICP-MS system to determine metal concentrations such as: Fe, Mn, Sn, Pb, Cd, Se, Hg and Cr (Rui *et al*., 2008a and 2008b). All experiments were repeated in three replications.

Measurement of plant height, leaf number, and fresh weight of lettuces: The height of plants and leaf number of lettuces were determined at the first day and the days after transplanting (DAT) includes the seventh, the fourteenth, the twenty-first and the thirty-five days, respectively, and before GA_3 spraying. The height of lettuces was measured from the growing point to cotyledon node while leaf number was calculated by counting leaf by leaf. After treating with GA_3 for 35 days, lettuce plants were harvested (removal of roots), and then were immediately weighted for fresh weight.

Measurement of nutrient contents in lettuces: The shoots were dried at 65°C for 24-36 hour until constant weight by a fan-forced oven, and then were ground to a fine powder using a high-speed pulverizer to determine nutrient contents. About 30 mg of lettuces were treated by 6 mL HNO_3 (98%) for 24h. Then 3 mL H_2O_2 was added into each sample before digesting at 180°C for 4-5 h until 1 mL solution remained by electrical plate. The residue was diluted with deionized water to 10 mL, nutrient contents were determined using inductively coupled plasma mass spectrometer (The Agilent 7900 ICP-MS).

Determination of gibberellic acid GA_3 accumulated in lettuces: For determination of GA_3 concentration in lettuces, samples were collected at the dates, including the beginning transplanting, the date 7, the date 14th, the date 21th, and the date 35th (harvest day). Approximately 300 mg of lettuce samples (fresh and fine powder) were treated by 3,000 µL of 2-propanol/water/concentrated HCl (2:1:0.002, v/v/v) solution, then shaken at 4°C for 30 min. Afterwards, 6,000 µL of dichloromethane was added and agitated for another 30 min at 4°C before centrifugation at 4,000 rpm for 10 min. There were two phases forming after centrifugation, and lower layer was concentrated by the nitrogen flow for 1 hour, and then dissolved in 1 mL MeOH. The samples were then filtered through 0.22 µm pore size PTFE membranes before injection and analyzed by the LC-ESI-MS/MS system (Qingfeng *et al*., 2014).

Data analysis: The one-way analysis of variance (ANOVA) was applied to analyze the data using SPSS 22.0 software. The means are averaged from three replicates and the standard derivations with a confidence interval of 95% ($p < 0.05$) in all cases.

RESULTS

The mean concentrations of metals in the experimental soil: The mean concentrations of metals (Fe, Mn, Sn, Pb, Cd, Se, Hg and Cr) in the experimental soil were shown in Table 1. Fe was the highest among the metals with a mean value of 63.2736 (mg/kg) recorded in the study soil. The second position belonged to the mean concentration of Mn, at 25.2431 (mg/kg). The concentrations of Pb and Cr elements ranged in the third and fourth positions, at 2.4314 (mg/kg) and 2.1674 (mg/kg), respectively. Particularly, Sn concentration was recorded in 1.0565 (mg/kg) whereas the mean values of Cd, Se and Hg concentrations in the experimental soil were 0.2277 (mg/kg); 0.0956 (mg/kg) and 0.0897 (mg/kg), respectively. This indicated that there was a difference in metal contents in the soil using for experiments.

Table 1. Metal concentrations in the experimental soil.

	Fe	Mn	Sn	Pb	Cd	Se	Hg	Cr
Concentrations (mg/kg)	63.2736	25.2431	1.0565	2.4314	0.2277	0.0956	0.0897	2.1674

The effect of GA_3 on the growth of *Lactuca sativa* L.: Figure 1 illustrates the influence of GA_3 on the growth of lettuces via plant height and the number of leaves during the 35 days of experiment. It is interesting to note that the plant height was obviously lower in plants treated with 100 (mg L^{-1}) during the experimental process in comparison with those of the controls and the lower concentrations of GA_3. No significant difference was found in the plant heights of lettuces between the control and the treatments in 10, 30 and 50 (mg L^{-1}) GA_3 at 7 DAT. Nevertheless, the effects of GA_3 concentrations on the plant height were clearly determined from the 14 DAT. It is obviously from the Figure 1A, the plant heights of lettuces exposed to 10, 30 and 50 (mg L^{-1}) GA_3 concentrations were higher than those of the control and plants exposed with 100 (mg L^{-1}) GA_3 at 14 DAT and 21 DAT. Particularly, there was no significant different between the lettuces treated with 10, 30 and 50 (mg L^{-1}) GA_3 at 14 DAT as at 21 DAT. After 35 days of experiments, the plant heights were 30.1 cm, 29.5 cm, and 27.5 cm in the plants treated with the concentrations of 30, 10, and 50 (mg L^{-1}) GA_3, respectively, which were taller than those of the controls (25.2 cm) while the heights of lettuces in the treatment of 100 mg L^{-1} GA_3 were 14.6 cm, were lower than those of the controls.

It is clear from Figure 1B, there were no differences in the leaf numbers of lettuces between the GA_3 treatments and the control until the 14 DAT. The influences of GA_3 exposures on the leaf number were significant at 21 DAT, wherein, the numbers of leaves of plants treated with 10, 30, and 50 (mg L^{-1}) GA_3 were higher than the 100 (mg L^{-1}) GA_3 treatment and the control. Nevertheless, no considerable differences were found in the leaf number between the other three GA_3 exposures. The highest numbers of leaves were 9.28 (leaves/plant) in the plants exposed to 30 (mg L^{-1}) GA_3, followed by 8.76 leaves/plant and 8.53 leaves/plant in the treatments of 10 and 50 (mg L^{-1}), respectively, which were significantly higher than the leaf numbers of the controls (7.67 leaves/plant) and the 100 (mg L^{-1}) GA_3 treatment. This demonstrate that low concentrations of GA_3 promote an increase in the leaf number of lettuces, whereas high concentrations of GA_3 constrain the development of lettuce leaves.

The noticeable differences were reported in the fresh weights of *Lactuca sativa* L. between the control and GA_3 treatments over 35 experimental days. As can be seen from the Figure 2, the highest fresh weight of lettuces was 30.08 (g/plant) at the 30 (mg L^{-1}) GA_3 treatment, which was 37.67% higher than the fresh weight of the controls (18.75 g). The following were 29.54 g/plant and 21.50 g/plant in plants treated with 10 and 50 (mg L^{-1}) GA_3, respectively, which were 36.53% and 12.79% higher than the lettuces without exposure of GA_3, respectively. Particularly, a figure of 14.58 g/plant was obtained in the fresh weight of lettuces in the treatment of 100 mg L^{-1} GA_3, which were 22.24% lower than those of the controls. The results illustrated that different concentrations of GA_3 showed the different influences on the fresh weight of lettuces, wherein, the 30 mg L^{-1} GA_3 is considered as the most suitable concentration for the growth of lettuces.

The effect of GA_3 on the nutrient contents and heavy metals in *Lactuca sativa* L.: Table 1 shows the influence of GA_3 application on nutrients (Fe and Mn) and heavy metals (Sn, Pb, Cd, Se, Hg, and Cr) in lettuces after 35 days of experiments. There was a noticeable difference in the nutrient contents of lettuces between control and GA_3 treatments. Obviously, the contents of Fe in lettuces treated with 10, 30, 50, and 100 (mg L^{-1}) of GA_3 concentrations were fluctuated in the scope of 0.0014-0.0090 (mg kg^{-1}), were significantly lower than those of the controls (33.1813 mg kg^{-1}). Similarly, Mn contents in plants sprayed by GA_3 concentrations (10-100 mg L^{-1}) were changed between 0.0001 and 0.0025 (mg kg^{-1}), which were smaller than the lettuces without GA_3 treatments (13.9890 mg kg^{-1}). It is interesting to note that, the contents of nutrients such as Fe and Mn were decreased with the increase of GA_3 concentrations from 10 to 100 (mg L^{-1}).

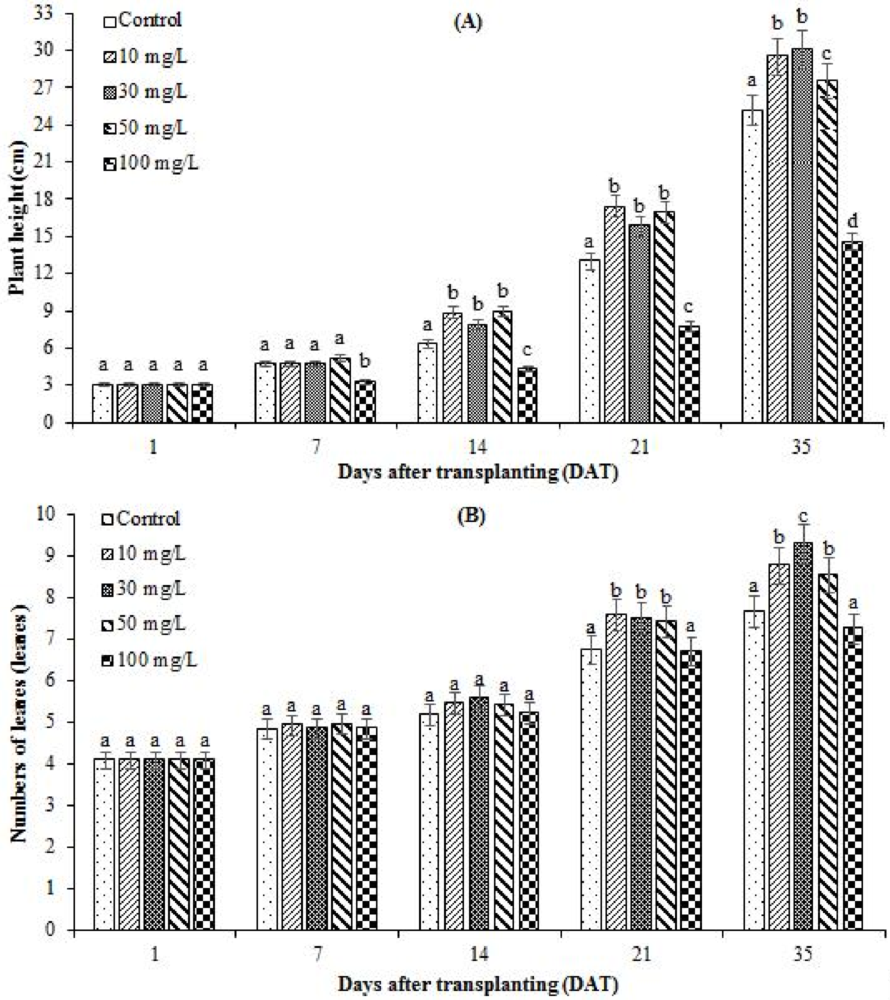

Figure 1 – The effect of GA_3 on the height (A) and leaf numbers (B) of *Lactuca sativa* L.
(Different letters on the same DAT show significant differences at $p < 0.05$ level between GA_3 treatments and control)

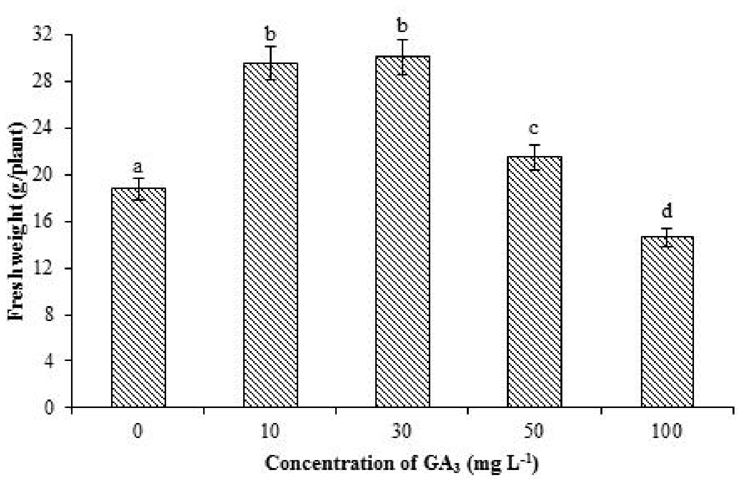

Figure 2 – Effects of GA_3 on the fresh weight of *Lactuca sativa* L.
(Different small letters indicate significant differences at $p< 0.05$ level between GA_3 treatments and control)

Table 1 – The effect of GA_3 application on the contents of nutrients and heavy metals in *Lactuca sativa* L.

GA_3 (mg L^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Sn (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Se (mg kg^{-1})	Hg (mg kg^{-1})	Cr (mg kg^{-1})
Control	33.1813^{a}	13.9890^{a}	0.2729^{a}	1.6489^{a}	0.0812^{a}	0.0125^{a}	0.0130^{a}	0.5189^{a}
10	0.0090^{b}	0.0025^{b}	0.0045^{b}	0.0045^{b}	0.0005^{b}	0.0025^{b}	0.0060^{b}	0.0208^{b}
30	0.0080^{b}	0.0015^{b}	0.0020^{b}	0.0005^{c}	0.0002^{b}	0.0020^{b}	-	0.0194^{b}
50	0.0069^{b}	0.0008^{b}	0.0015^{b}	-	-	0.0010^{b}	-	0.0169^{b}
100	0.0014^{c}	0.0001^{b}	0.0001^{b}	-	-	0.0001^{b}	-	0.0108^{b}

(Different small letters on the same column present the significant difference at $p < 0.05$ level between GA_3 treatments and control; "-": none detection)

As can be seen from the Table 1, the considerable difference was reported on the contents of heavy metals namely Sn, Pb, Cd, Se, Hg, and Cr in lettuces (*Lactuca sativa* L.) exposed to GA_3 treatments in comparison with those of the controls. The results showed that Sn, Se, and Cr contents in lettuces sprayed with GA_3 treatments were decreased among with the increase in concentrations of GA_3 application, and were remarkable lower than those of the controls. Se contents in lettuces exposed to GA_3 were changed between 0.0001 and 0.0025 (mg kg^{-1}), were 80-99% lower than those of the controls (0.0125 mg kg^{-1}). The Cr contents in lettuces at the GA_3 treatments fluctuated from 0.0108-0.0280 (mg kg^{-1}), was 96-98% lower than the plants without GA_3 (0.5189 mg kg^{-1}). Sn contents were found at 0.0001-0.0045 mg kg^{-1}, were 99% lower than those of the control. It is interesting to note that, Pb and Cd contents in lettuces were decreased by 10 and 30 (mg L^{-1}) of GA_3 treatments, however, these heavy metals were not found in lettuces treated with 50 and 100 (mg L^{-1}) of GA_3. Particularly, the contents of Hg were found at 0.0060 mg kg^{-1} in the 10 (mg L^{-1}) of GA_3 treatment which were 53.9% lower than those of the controls (0.0130 mg kg^{-1}), but were without appears in plants treated with higher concentrations of GA_3 (30, 50 and 100 mg L^{-1}). These results illustrate that the decrease in the content of heavy metals in lettuces exposed with GA_3 in comparison with those of the control.

The bio-accumulation of GA_3 in *Lactuca sativa* L.: Figure 3 reveals the GA_3 contents in lettuces after 35 days exposed to plant growth regulator (GA_3). No significant differences were reported in the concentrations of GA_3 in lettuces between the control and GA_3 treatments at the first transplanting and 7 DAT. As can be seen from the Figure 3, GA_3 contents in lettuces were increased among with the time of experiment from the 14 DAT to the 35 DAT. The contents of GA_3 were found at 0.079; 0.090; 0.114, and 0,130 (mg kg^{-1}) in the lettuces treated with 10, 30, 50, and 100 (mg L^{-1}) GA_3, respectively, which were higher than those of the controls (0.075 mg kg^{-1}) at the 14 DAT. Obviously, GA_3 contents were increased to 21.7%; 38.5%; 55.56%, 63.6% in plants exposed to GA_3 treatments including concentrations such as 10, 30, 50, and 100 (mg L^{-1}) in comparison with those of the controls at the 21 DAT. Particularly, after 35 days of experiments, the highest content of GA_3 was 0.265 (mg kg^{-1}) in lettuces exposed with 100 (mg L^{-1}) GA_3; following, was 0.210 (mg kg^{-1}) upon 50 (mg L^{-1}) GA_3 treatment; then, were 0.145 and 0.100 (mg kg^{-1}) in the exposures of 30 and 10 (mg L^{-1}) GA_3, respectively; and, the last was 0.065 (mg kg^{-1}) in the control. These data illustrated that the higher concentrations of GA_3 treatment use, the more their accumulations in lettuce samples.

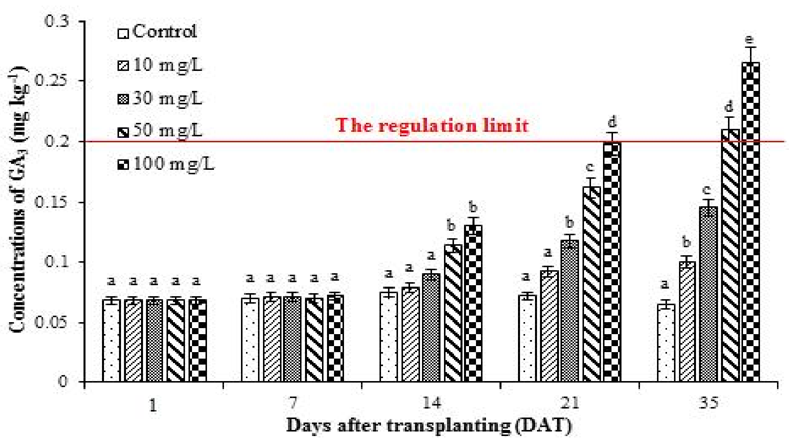

Figure 3 – The remained content of GA_3 accumulating in *Lactuca sativa* L.
(Different small letters on the same DAT show significant differences at $p < 0.05$ level between GA_3 treatments and control)

DISCUSSION

The application of GA_3 on lettuces (*Lactuca sativa* L.) were reported in the previous studies. Georgeo *et al.* (2014) indicated that, lettuces were increased in plant heights, leaf numbers, and the fresh and dry weights under GA_3 treatments. According to Taslima (2015), the tallest plant heights of lettuces were 18.16 cm found in the treatment of 50 mg L^{-1} GA_3 whereas the shortest of lettuces were reported in those of the control (15.57 cm). However, the highest yield of lettuces was 29.05 tons/ha in plants treated with 25 mg GA_3 while those of the control was only 16.27 tons/ha. In the other study, Alessandro *et al.* (2019) evaluated the effects of GA_3 on lettuces via the growth and yield parameters. Different concentrations of 0, 10^{-8}, 10^{-6}, and 10^{-4} (M) GA_3 were added into the floating system's mineral solution and were tested for 22 days. The lettuces were stimulated in the growth and were enhanced in the yield upon the exposures of 10^{-8} and 10^{-6} M GA_3 concentrations, especially, the highest leaf number per lettuce was obtained by treated to 10^{-6} (M) GA_3. Our data are in agreement with these studies that gibberellic acid (GA_3) was obviously impacted on the growth of lettuces, wherein, low concentrations of GA_3 was positive affected on the plant height, leaf number and biomass of lettuces. This is the basic to choose the suitable concentration of GA_3 applying to increase the productivity and efficiency in other vegetables and agricultural crop production.

In addition, several reports were studied the effects of GA_3 on the nutrients as well as the accumulation of heavy metals in agricultural plants. Akman (2009) indicated that the highest contents of Fe and Mn in wheat were found in the treatment without GA_3, whereas GA_3 application increased Fe and Mn contents in barley compared to the control. In the other study, Mukhtar (2008) found that 100 (mg L^{-1}) of GA_3 decreased Fe and Mn contents in *H. sabdariffa* in comparison with those of the controls. However, according to Hassan *et al.*, (1979), GA_3 was reported as the plant growth regulator enhancing to uptake of nutrients as Fe and Mn in corn plants. These data indicated that Fe and Mn contents in different plants were different impacted by GA_3 application, especially, the decrease of Fe and Mn contents in lettuces in comparison with those of the controls, which need further study to limit the impacts on the quality of vegetables.

The accumulation of Pb and Cd were found in lettuces (*Lactuca sativa* L.) watered with the wastewater from the Quetta city (Abdul *et al*., 2011). According to Mamdouh and Osam (2018), Pb and Cd were uptaken and accumulated by lettuces grown on a metal-contaminated soil. Chang *et al*. (2013) reported lettuces uptake and accumulate heavy metals, namely Cd, Hg, Pb, and Cr in leaves from the soils collected in the Pearl river Delta, South China. These data indicate that lettuces could uptake and accumulate heavy metals in theirs leaves, which associated with potential health risks to human. However, our study results showed that Sn, Pb, Cd, Hg, Se, and Cr were presented in low concentrations in lettuces exposed to GA_3 compared with those of the plants without GA_3 treatment while the same properties of soil present in Table 1 using for experiments. It might the application of GA_3 decrease the uptake of heavy metals in lettuces, which is needed to further investigate to limit the potential risks impact on consumers and ecological environment as well as to study and application of GA_3 in limiting heavy metals' absorption in other vegetables and agricultural crops.

The residues of GA_3 in fruits and vegetables might be the potential risks influence to consumers' health (Gehan *et al.*, 2015). According to the Maximum Residue Limits (MRLs) of the EU, the safety limit of gibberellin residues in vegetables was 0.20 mg kg^{-1} (EU, 2010). In addition, several countries, such as Japan and U.S.A have regulated that a content of 0.20 mg kg^{-1} was considered as the maximum residue limit of gibberellins in vegetables (Yuan and Xu, 2002). This illustrated that the residues of GA_3 in lettuces treated with high concentrations of GA_3 (50 and 100 mg L^{-1}) were higher than the regulations of the EU, U.S.A and Japan. On the other hand, some researchers indicated that the application of agrochemicals, especially, plant growth regulators might be lead to environmental pollution, serious affect to human health and animals (Celik and Kara, 1997; Sørensen and Danielsen, 2006; Ismail *et al*., 2007; Erin *et al*., 2008). This suggests that high concentrations of GA_3 (50 and 100 mg L^{-1}) were applied in vegetable production cause the high ratios of GA_3 contents accumulate on vegetables, which necessary to further study for improving the efficiency of vegetable production using plant growth regulator (GA_3) as well as minimize their impacts on human health and the ecological environment.

Conclusions: In summary, GA_3 had significant effect on the growth, nutrient elements, and the absorption of heavy metals in lettuce (*Lactuca sativa* L.). Low concentrations of GA_3 (10 and 30 mg L^{-1}) increased the plant height, leaf number and fresh weight of lettuces whereas the opposite results were reported in high concentration of GA_3 treatment (100 mg L^{-1}). We found that nutrient contents (Fe and Mn) in lettuces treated with GA_3 were significant decreased in comparison with those of the controls. In addition, the treatment of GA_3 reduced the absorption of heavy metals (Sn, Pb, Cd, Se, Hg, and Cr) in lettuces, which may limit the potential risk impacts on human health. However, the higher concentrations of GA_3 treatment were used, the more of its accumulation was found in lettuce samples. High contents of GA_3 were found in lettuces treated with 100 and 50 (mg L^{-1}) GA_3 (0.265 and 0.210 mg kg^{-1}, respectively), which were higher than the regulations of the EU, U.S.A and Japan, and might be affected on the health of consumers. To the best of our knowledge, this is the first investigation on the influences of plant growth regulator (GA_3) on nutrient contents and heavy metals' absorption, and the accumulation of GA_3 applying in high concentrations in lettuces. However, further studies are needed for improving the efficiency of vegetable production using plant growth regulator as well as minimize their impacts on consumers' health and the ecological environment.

Acknowledgement: This study was supported by the Vietnam Academy of Science and Technology, grant number: TD NDTP.01/19-21 and QTHU01.01/20-21; and National Research, Development and Innovation Office, Hungary, grant number: K124430.

Conflict of interest: No competing financial interests between the authors.

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