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RESEARCH ARTICLE

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Effect of gamma irradiation on some active constituents and metabolites of *Cichorium pumilum* Jacq.

ABSTRACT:

A biotic stress mostly controls medicinal compounds productivity in the plant. This study was carried out to investigate the effect of gamma irradiation on some active constituents in shoot and root of *Cichorium pumilum* (Chicory). Irradiated seeds with 20, 40, and 80 gray (Gy) by cobalt 60 gamma irradiation were cultivated under normal field conditions. Results showed that the plant dry weight was stimulated at the first growth stage and inhibited at the following stages. Photosynthetic pigments (chl. a & b) were inhibited but carotenoids increased as a defensive effect to the increase in gamma doses. Alkaloids content was twice the phenolic and several times the saponins content under the control plant. Saponin content in the plant root increased with all gamma doses and the highest increase (69.5%) was at 40 Gy compared to the control. Likewise, the total alkaloids content was increased by 49.7% at the 40 Gy in the plant shoot. Accumulation of alkaloids was more sensitive to gamma radiation in shoot than in root. The 40 Gy dose enhanced accumulation of total phenolic compounds in root. Increasing gamma dose to 80 Gy inhibited the accumulation of three secondary metabolites in plant shoots and roots especially the alkaloids content that was reduced per plant to the half. The study suggested 40 Gy dose as an efficient dose to induce the metabolism and accumulation of active compounds of Chicory plants. In addition, it certified the vegetative stage which used as a human food to produce such compounds from the total plant (shoots and roots).

KEY WORDS:

Chicory, *Cichorium pumilum*, Abiotic stresses, Gamma Irradiation Secondary metabolite, Alkaloids, Phenolic compounds, Saponin.

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INTRODUCTION:

The natural environment for plants is composed of a complex set of abiotic and biotic stresses. Different types of abiotic stresses are known to have strong impact on morphological and development of plants. Plant responses to these stresses are equally complex (Cramer *et al*., 2001; Blagojevic, 2015). In the last decade, gamma irradiation has been drawn the attention as an abiotic stress and a new rapid method to improve the qualitative and quantitative characters of many plants (Charbaji and Nabulsi, 1999).

Gamma rays belong to ionizing radiation and interact to atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the species, plant growing stage and radiation dose (Neelam *et al*., 2014) and the effects include changes in the plant cellular structure and metabolism (Kim *et al.,* 2004).

Mohamed and El Shimi (2014) pointed out all doses of gamma used in his research led to a decrease in dry weight. Dry weight has an inverse relationship with the degree of gamma dose from 5 to10 Gy. The gamma radiation had a significant effect on most of the characteristics of the plant, including the dry weight of the shoot, and increase was continued up to 100 Gy, then it decreased in the high doses.

Cichorium pumilum (commonly known as Chicory) is a weed grows naturally associated with clover fields during fall, winter and spring seasons. *Cichorium pumilum* is an annual herbaceous plant of the dandelion up to 90 cm, all plant parts exudate a milky latex. It is preferable and eaten by the Egyptian farmers with cheese as a common Egyptian meal (Abou-Zeid, 2015). Chicory is known by its medicinal importance. However, according to EMA (2012) traditional use of chicory roots includes the relief of symptoms related to mild digestive disorders (such as feeling of abdominal fullness, flatulence, and slow digestion) and temporary loss of appetite. Krylova *et al.* (2006) reported that *C. pumilum* had anti- hepatoxic effects (decreases glycogen content, cell necrosis and increases the number of cells with pronounced protein synthesis activity) induced hepatic damage. Hazra *et al*. (2002) report that extract root of Cichorium has a restraining influence on the type of tumors in mice. Pushparaj *et al.* (2007) stated that in Turkey traditional medicine used Roots decoction to protective of Cancer and kidney stones. Vohra (2004) pointed out that Chicory has been listed as one of the 38 plants that are used to prepare Bach flower remedies, a kind of alternative medicine. The medical importance of *Cichorium* sp. lies mainly in the photosynthetic pigments (chlorophyll a, b and carotenoids) and the secondary metabolites alkaloids, phenolic compounds and saponins of its edible parts. The content of these important metabolites evaluates its economic utilization as a complementary food and a source of many medicinal compounds. Aqil *et al*. (2006) reported that the plant acquires strong free radical scavenging activity. The phytochemical analysis of plant extracts indicated the presence of the major phyto compounds, including phenolic compounds, alkaloids, glycosides, flavonoids, and tannins. The phenolic concentrations in the plant ranged from 28.66 to 169.67 mg/g of dry plant extract and there is a fair correlation between antioxidant/free radical scavenging activity and its phenolic content**.**

Saponin have various effects attributed to a diverse range of properties, some of which induce both beneficial and detrimental effects on human health such as pesticides, insecticidal and molluscicidal activity, allelopathic action, anti-nutritional effects, sweetness and bitterness, and as phyto-protectants those defend plants against attack by microbes and herbivores (Hostettmann, 1991). Also, saponin is useful medically in controlling cholesterol, decreasing incidence of heart diseases and protection from cancer (Oakenfull and Sidhu, 1990). The phenolic compounds play important physiological and ecological roles, being involved in resistance to different types of stresses (Ayaz *et al*., 2000).

All parts of this plant have major medicinal importance because of the presence of several medicinally important compounds such as sesquiterpene, lactones, flavonoids, alkaloids, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, inulin, saponins and tannins (Nandagopal and Ranjitha 2007). The bitter compounds in the plant namely, lactucin, lactucopicrin, and the guaianolide sesquiterpenes, isolated from aqueous root extracts of chicory were concluded to be the antimalarial components of the plant. Lactucin and lactucopicrin completely inhibited the HB3 clone of strain Honduras-1 of Plasmodium falciparum at concentrations of 10 and 50 μg/ml, respectively (Bischoff *et al.,* 2004).

The aim of the present study is to investigate effect of gamma irradiation on some secondary metabolites having a medicinal importance in the shoot and root of *C. pumilum* at the active growth (vegetative) stage.

MATERIAL AND METHODS:

Cichorium pumilum is a Mediterranean plant species belongs to the subfamily Cichorioideae, Asteraceae. Seeds of the plant were obtained from Weed Research Center, Sakha, Kafr El-Sheekh. Chicory seeds were irradiated with gamma rays at (0, 20, 40, and 80 Gy) emitted from cobalt 60 source at room temperature. Irradiation process was performed at the National Institute of Standards (NIS), Giza, Cairo, Egypt. Seeds were germinated in plastic pots of 20 cm diameter and 30 cm depth in the green house of Menoufia University, Egypt. The seeds were irrigated with tap water once every 3 days or whenever needed until the growing of seedling. The plant at vegetative stage was harvested (after 78 days). The plants were separated into shoot and root and dried in an oven at 50 °C to constant weight. The plant shoots and root dry weights were recorded, and the dried materials were powdered and preserved in paper bags until using.

Estimation of photosynthetic pigment:

The quantitative values of pigments (chl. a, b, and carotenoids) were extracted and measured by using absorption proposed by Welburn (1994).

Estimation of saponin:

Saponin was estimated quantitatively by the method described by Hiai *et al*. (1975). A standard curve by cholesterol was constructed as in the previous steps and used for the determination of the content of saponin (mg/g d.wt).

Determination of total alkaloids:

Total alkaloids were extracted, precipitated and measured quantitatively according to the method described by Harbone (1973). Alkaloids content was calculated and expressed as (mg/g dry) weight of the plant samples.

Estimation of total phenolic compounds:

Total phenolic content was estimated quantitatively using the method described by Jindal and Singh (1975). A standard curve was prepared by using different concentrations of gallic acid and used for the determination of total phenolic compounds content (mg/g d.wt).

Data statistical analyses:

One-way ANOVA analysis was used to signify treatments variation. Correlation coefficients between the recorded variables were evaluated. All the statistical methods were according to (Bishop, 1983), while the analysis was carried out by IBM SPSS statistical package.

RESULTS AND DISCUSSION:

The plant weights:

The plant dry weight result was represented in table 1 and indicated greater shoot than root weight at all growth stages. All gamma doses decreased shoot dry weights at all growth stages while they decreased root dry weights at flowering and fruiting stages but at the earlier stages seedling and vegetative stages gamma doses increased the plant dry weight in comparison with the control. The maximum gamma dose (80 Gy) greatly inhibited the shoot and root dry weights. Mohamed and El Shimi (2014) pointed out that plant dry weight in their study has an inverse relationship with the increase of gamma dose from 5 to10 Gy.

Table 1. The shoot and root dry weight (g d. wt.) of Chicory ± the SD under the effect of 0-80 Gy gamma irradiation during the different growing stages.

		Gamma ray (Gy)			
Stage	Organ	0	20	40	80
Seedling	Shoot	0.028 ± 0.011	0.019 ± 0.003	0.017 ± 0.001	0.018 ± 0.005
	Root	0.012 ± 0.003	0.016 ± 0.003	0.016 ± 0.004	0.015 ± 0.002
Vegetative	Shoot	0.325 ± 0.024	0.322 ± 0.058	0.289 ± 0.066	0.191 ± 0.054
	Root	0.246 ± 0.062	0.250 ± 0.033	0.263 ± 0.020	0.257 ± 0.006
Flowering	Shoot	0.875 ± 0.314	0.714 ± 0.067	0.723 ± 0.111	0.579 ± 0.124
	Root	0.638 ± 0.153	0.603 ± 0.054	0.416 ± 0.093	0.320 ± 0.051
Fruiting	Shoot	1.079 ± 0.416	0.820 ± 0.258	0.791 ± 0.318	0.839 ± 0.102
	Root	0.779 ± 0.113	0.808 ± 0.211	0.720 ± 0.074	0.583 ± 0.103

Plant photosynthetic pigments:

The chlorophyll a, b, and carotenoid contents in *C. pumilum* leaves (Fig. 1) varied slightly in response to the effect of the different gamma doses at the vegetative growth stage. At vegetative stage the chlorophyll b content significantly decreased in plant leaves in all gamma radiation doses comparing with the control and the maximum decrease, by 7%, was recorded at 20 Gy. Under all gamma doses chlorophyll a was relatively higher than chlorophyll b and the increase in gamma doses increased the difference between them due to the more inhibition in chlorophyll b synthesis. Jan *et al.* (2013) found that plant photosynthetic pigments were stimulated under low gamma doses and then reduced with increasing gamma radiation. The carotenoids synthesis at the vegetative stage was increased with the increase of the used gamma doses and the highest carotenoids content by 80Gy was up to 102%. Kovacs and Keresztes (2002) investigated the effect gamma dose on plant and observed that gamma dose led to increasing carotene content as a way of gamma stress defense.

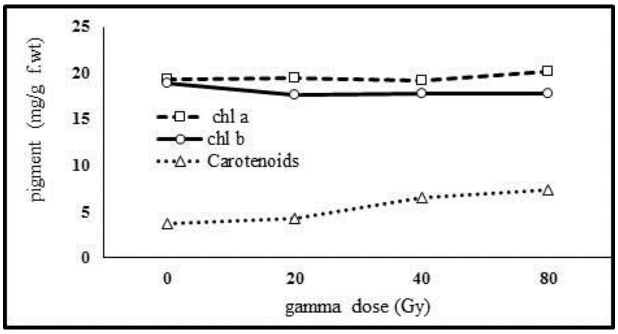

Fig. 1. Effect of gamma irradiation doses on the photosynthetic pigments (mg/g f.wt) of Chicory.

Saponin content:

Figure 2 shows the saponin content of *C. pumilum* under influence of different doses of gamma irradiation at the vegetative growth stage. In general, most doses used in the study caused increased saponin content. All radiation doses caused a significant effect on the saponin content ($P \leq 0.01$) in both plant shoot and root. The shoot saponin content was greater than root content under all gamma ray doses except 40 Gy dose.

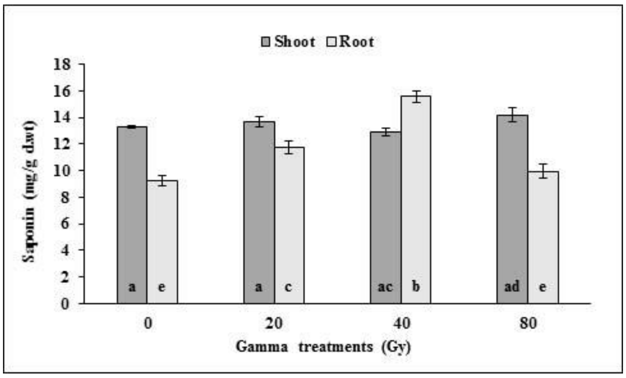

Fig. 2. Effect of gamma irradiation doses on saponin contents (mg/g d.wt) of Chicory Error lines represent the SD of three replicates of the mean independent experiments. Columns with the same letter are not significantly different (at $p \leq 0.05$).

In the shoot, the application of 80 Gy doses of gamma irradiation show maximum induction in saponin contents and led to an increase up to 5% compared to control, while 40 Gy dose had a negative effect and led to a slight decrease. Likewise, in the root, all gamma radiation doses especially low ones had a positive effect on saponin content of Chicory and 40 Gy recorded the highest increase in saponin content where it was up to 69.5% of the control. According to the obtained results in the study, we can conclude that the use of gamma doses up to 80 Gy was enhancing of saponin metabolism in Chicory root. These results are in harmony with those obtained by Mohamed (2009). This response may be associated with gamma ray stress tolerance in the Chicory because of the modulation of enzymes activity and defensive pathways as found by Zhang *et al*. (2011).

Alkaloid content:

The statistical analysis of the obtained data of alkaloid compounds content showed that gamma ray treatments caused highly significant variations ($p < 0.01$) in alkaloid content of *C. pumilum* shoot and root (Fig. 3). Alkaloids content was greater in root than in shoot of the plant and it was about double under control and low doses of gamma rays reduced this ratio of root to shoot content. On the contrary, the 80 Gy dose greatly inhibited the alkaloids content of shoot that caused many times root content compared to shoot content. Alkaloids content in shoot increased gradually at low doses and 40 Gy recorded the maximum increase of alkaloids in Chicory that was by 49.7% of the control. Meanwhile, doubling the dose to 80 Gy caused a severe inhibition of alkaloids metabolism in shoot of the plant. These results agree with the findings of Benslimani and Khelifi (2014) that gamma rays had positive effects on alkaloid content in the *Datura innoxia*. In contrast, all the used gamma rays doses had relatively inhibited the accumulation of alkaloids content in the plant root but variation with the effect of the different doses was not significant. Similar varied results in content and profile of alkaloids in *Emblica officinalis* were also obtained by Khattak (2013).

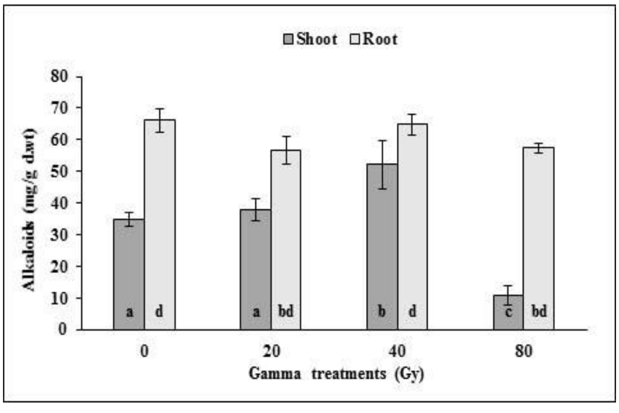

Fig. 3. Effect of gamma irradiation doses on alkaloid content (mg/g d.wt) of Chicory Error lines represent the SD of three replicates of the mean independent experiments. Columns with the same letter are not significantly different at ($p \leq 0.05$).

Also, the remarkable accumulation of the alkaloid compounds in the plant root than in shoot showed that most treatment induced translocation of alkaloids compounds from shoot to root rather increase their metabolism in shoot especially under the highest treatment (80 Gy). Several studies such as those of Kim *et al*. (2006) and Mohajer *et al*. (2014) report that gamma irradiation significantly affects the alkaloid content of different organs in various plants. It is also important to note that 40 Gy induced both metabolism of alkaloids in shoot and their translocation from shoot to root leading to high alkaloids content in the two organs.

Total phenolics:

The used doses of gamma radiation led to variable effects on the phenolic compounds content in the Chicory shoot and root, but most variations were not significant (Fig. 4). In the shoot, the recorded data indicated that the used gamma rays had an inhibitory effect on the production of phenolic compounds. The lowest phenolic compounds content was evident with the 20 Gy dose which caused 13.3% decrease compared to the control, followed by treatment with 80 and 40 Gy doses which led to 4.1% and 0.9% decreases of phenolic compounds, respectively compared to the control. This showed correlative inhibition of gamma-ray doses stress to the phenolic compounds metabolism in shoot.

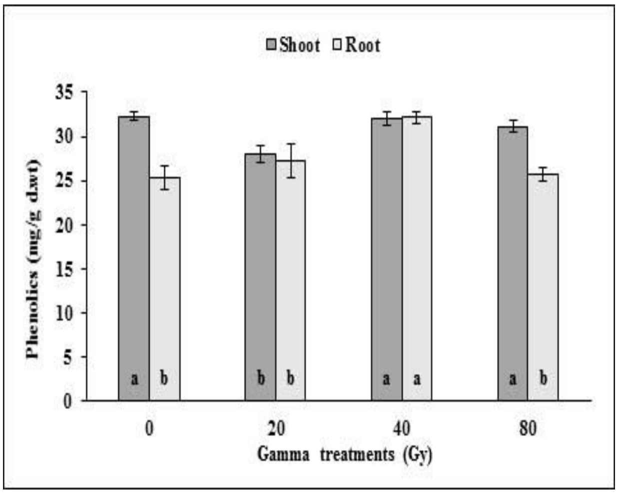

Fig. 4. Effect of gamma irradiation doses on total phenolic compounds (mg/g d.wt) of Chicory. Error lines represent the SD of three replicates of the mean independent experiments. Columns with the same letter are not significantly different (at $p \leq 0.05$).

In the root, contents of phenolic compounds were lower than those of shoot under all the used gamma doses except 40 Gy. All gamma doses induced increases in root phenolic compounds content compared with control and its highest content was due to at 40 Gy and was higher by 26.8 % in comparison with the control. On the contrary the highest level of gamma dose (80 Gy) caused a slight decrease in the phenolic compounds in the plant root but its value was still higher than that of the control. The responsive increase in root content of phenolic compounds was due to induction of their translocation to root which was remarkably inhibited by the highest gamma dose (80 Gy). These results agree with the results of Mohamed (2009) who reported an increase in the amount of phenolic compounds that was gradually with the increase in gamma irradiation dose and the extreme increase was noticed at 40 Gy. The increase in the phenolic compounds might be attributed to the release of phenolic compounds from glycosidic components and increase the activity of larger phenolic degradation into smaller ones by irradiation as was reported by Stajner *et al.* (2007). Also, Oufedjikh *et al.* (2000) pointed out that gamma irradiation increases the activity of phenylalanine ammonia-lyase which is responsible for the synthesis of polyphenolic acids. The result recommended 40 Gy for the production of greater amounts of phenolic compounds especially when both shoot and root contents were considered.

The Correlation between the studies secondary metabolites:

The relationship between the secondary metabolite in *C. pumilum* under the effect of different doses of gamma-ray was represented in table 2. Alkaloids in shoot significantly (r < 0.01) and positively correlated with saponin and phenolic compounds of root at but negatively with shoot saponin positively. Shoot phenolic compounds correlated with alkaloid in root and negatively correlated significantly with saponin in shoot (r < 0.01). Saponin content in root correlate significantly and positively with phenolic in root but positively with saponin of shoot. The other relationships were non-significant. The previous relationship, especially the significant once, verified dependence of the root content from the three secondary compounds on the shoot content. This was due to the effected of the used gamma radiation doses on the translocation of metabolites rather than inhibition of their metabolism. The partition of the metabolites into the different plant organs was previously found to be affected by the different stresses such as gamma ray irradiation (Kim *et al.*, 2006; Mohajer *et al.*, 2014).

Table. 2. The correlation coefficients for the relationships between secondary metabolite in *Cichorium pumilum* under the different doses of gamma irradiation (0, 20, 40, and 80 Gy).

		alkaloids		phenolic		saponin	
		shoot	root	shoot	root	shoot	root
alkaloids	shoot	1					
	root	0.468	1				
phenolic	shoot	0.034	.571*	1			
	root	.670**	0.391	0.048	1		
saponin	shoot	-.799**	-0.494	-0.264	-.539*	1	
	root	.706**	0.102	0.018	.892**	-.562*	1

** Correlation is significant at r < 0.01. * Correlation is significant at r < 0.05.

Secondary metabolites per plant:

Figure 5 shows that content of secondary metabolites in the whole plant, in general, acquired the trend alkaloid > phenolic > saponin compounds indicating that alkaloid content represents the major medicinal compounds in comparison with phenolic compounds and saponin of chicory plant. Also, Gamma irradiation has a highly significant effect on the productivity of the medicinal compounds in the plant, especially alkaloid while change of saponin content was very close. The 40 Gy of gamma ray was the most suitable dose as it improved remarkably the studied secondary metabolites productivity (saponin, alkaloid and phenolic compounds) in chicory plant. The gamma ray increase of secondary metabolites yield was by enhancing the activity of certain key biosynthetic enzymes as reported by Vardhan and Shukla (2017). On the opposite, the dose up to 80 Gy led to the lowest content of those secondary metabolites. This dose reduced the plant content of alkaloids by more than 50% and with lower percentages for phenolic and saponin.

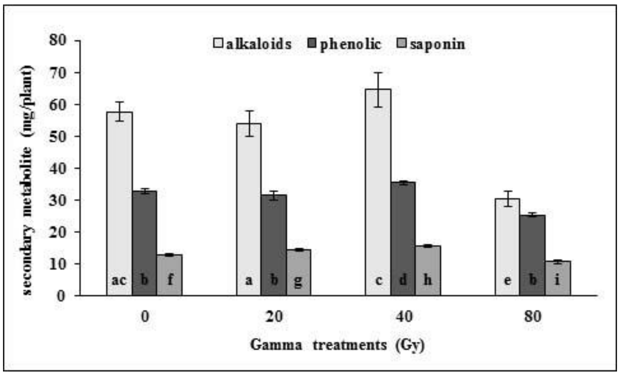

Fig. 5. Effect of gamma irradiation doses on secondary metabolite (mg/plant) of Chicory Error lines represent the SD of three replicates of the mean independent experiments. Columns with the same letter are not significantly different (at p ≤ 0.05).

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تأثير اشعة جاما على بعض المحتويات النشطة والأيضيات لنبات الشيكوريا

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يلعب الإجهاد دوراً هاماً في إنتاجية المركبات الطبية في النبات وقد أجريت هذه الدراسة للتحقق من تأثير الإجهاد بأشعة جاما على بعض المركبات في نبات الشيكوريا ولذلك تم تشعيع البذور بأشعة جاما عند جرعات 20 ، 40 ، 80 جراي ثم زراعتها في ظروف الحقل الطبيعية. أدت أشعة جاما إلى زيادة الوزن الجاف للنبات في المراحل الاولى من النمو وتراجعت هذه الزيادة في المراحل المتقدمة من النمو. كما ثبطت تخليق صبغات الكلورفيل أ، ب ولكن زادت نسبة الكاروتينات التى يستخدمها النبات كوسيلة دفاعية لمقاومة تأثير الزيادة في شدة الاشعاع. بشكل عام محتوى القوليدات كان ضعف الفينولات وأكثر بعدة أضعاف من محتوى السابونين في الكنترول. وكل الجرعات المستخدمة في الدراسة أدت إلى زيادة في محتوى السباونين في جذور النبات وسجلت الجرعة ٤٠ جراي أعلى نسبة زيادة وصلت إلى (69.5 %) مقارنة بالكنترول كذلك بالنسبة للقوليدات أدت تلك الجرعة (40 جراي) الى نسبة زيادة بلغت49.7 % داخل المجموع الخضري للنبات كما ان تراكم القوليدات في المجموع الخضري كانت اكثر حساسية لأشعة جاما منها في الجذور كذلك عززت الجرعة 40 جراي من تراكم الفينولات في جذور النبات. أدىزيادة التشعيع الى الجرعة ٨٠ جراي الى تثبيط تراكم محتوى المركبات الثانوية التي شملتها الدراسة خاصة محتوى القوليدات التي إنخفضت حتى النصف تقريبا في النبات ككل. تقترح الدراسة الاعتماد على جرعة 40 جراي كجرعة فعالة لحث عمليات الأيض وتكوين المركبات الثانوية في نبات الشيكويا كما تم توثيق المرحلة الخضرية التي تستخدم كغذاء كمصدر لإنتاج هذه المركبات في نبات الشيكوريا.