Biogenic selenium nanoparticles: their dual impact on spleen and growth in arthritic mice

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Selenium is known to be an important element for growth and splenic microenvironment regulation. About 5–10% of RA patients presented enlarged spleen along with low levels of selenium than the normal individuals. The current study aims to determine the effects of biogenic selenium nanoparticles (SeNPs) on the growth and spleen of healthy and arthritic mice. Biogenic SeNPs were evaluated for their adverse as well as therapeutic effects on the growth rate and splenic integrity of healthy and arthritic Balb/c mice. The tested doses of SeNPs significantly reduced the growth of mice along with splenomegaly in biosafety profiling while the treatment revealed dose-independent improvement in the growth of arthritic mice as compared to untreated arthritic control with no adverse effect on spleen. Concluding, selenium levels directly affect the growth of mice with no harm to splenic integrity in arthritic mice, however further investigation is required to develop a new method for evaluating the efficacy of Se sources.

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1. Introduction

Selenium (Se) is an essential trace element in humans and animals with broad range of biological activities like growth enhancing, immune modulation, anti-cancer, antiviral, antioxidant properties [1-4] metabolism of thyroid hormone and reproduction [5] and its deficiency can cause numerous diseases like liver diseases, cancer, heart disease and cataracts etc [2]. Selenium is well known essential micronutrient at trace concentrations but its excession may lead to selenosis. Soil and water are the natural sources of Se through which it enters food chain, plants receive Se from soil, which is its primary source in the diet of humans and animals. Deficiency of Se hinders the biosynthesis of seleno-proteins in humans leading to reduced activity of antioxidant enzymes [3, 6]

Selenium is known to be important element for growth [1] and its deficiency also reported to induce growth retardation in second generation of Se-deficient rats, but the exact growth decline mechanism remains unclear. It is suggested by various studies that Se deficiency affects the bone tissue in man. A significant correlation was observed between the collagen pyridinium crosslinks and urinary Se levels in healthy infants suggesting a link between Se status and bone integrity as its deficiency also played a role in Kashin-Beck osteoarthropathy, an endemic in Tibet and China [7]. The recommended daily Se intake for adult human body is about 50-70 μg/day [4, 6]. The toxicity of Se as supplement in arthritis treatment is under debate due to a minute difference between its toxicity and essentiality [4, 6, 8, 9]. RA patients have low levels of Se than the normal individuals suggesting Se supplements as the complementary medicine to treat RA [8].

For more than 60 years, intoxication of Se has been studied in animals and was named alkali disease in 1860, while in severe cases, necrosis of liver and central nervous system is involved. However, the most prominent symptom is growth retardation in young animals and is concluded by the American National Research Council in 1976 that growth retardation might be

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the top indicator of Se toxicity [10]. Various studies suggested an inverse relationship between the levels of Se and innate immune system of animals and humans. Reduced Se impairs innate immunity of host leading to greater susceptibility of viral and bacterial infections. Therefore, Se has a key role in development of immune organs and in regulating their innate immune responses [11, 12]. Spleen a peripheral lymphoid organ has a significant role in the immune system, as in chickens Aflatoxin B1, heat stress, and cadmium impaired immune function of spleen was improved by dietary Se. Selenium deficiency effects the function of lymph nodes and spleen which is the site of maturation and differentiation of multiple cells of immune system. Recent studies revealed that Se plays a significant role in regulating erythroid progenitors, stress and spleen microenvironment [12], as Se deficiency lead to decrease lymphocyte corpuscles, congested red pulp and disorganized red and white pulp areas of spleen [11]. Thus, Se is very important for the function and development of spleen [12]. A study conducted by [13] on lymphoid organ development proposed that Se deficient diets affects the development and growth of immune organs which leads to a decrease in number of splenocytes (phagocytes and lymphocytes) and spleen weight in Se-deficient chickens but the exact underlying molecular mechanism of Se-deficiency induced growth impairment remains unclear [11, 12].

Nanoparticle preparations have been developed for more effective dosing of Se [14]. Nano-red elemental Se, a new source having unique properties such as a high surface activity, large surface area, strong adsorbing ability, high catalytic efficiency, and low toxicity [1]. It is capable to scavenge free-radicals by improving the activity of seleno-enzymes [15, 16], and growth improvement along with the status of serum oxidants and retention of Se in-vivo. In-comparison with other Se species like se-methionine, selenite, se-methyselenocysteine, and se-yeast, the nano-selenium showed lower acute toxicity but increased the activities of selenoenzymes [16]. By using nanoparticles, it is possible to enhance the bioactivity and bioavailability of drugs and enable selective targeting of inflamed joints. It is confirmed by previous studies that the use of nanoparticles is feasible in treatment of Rheumatoid Arthritis [8] due to their low toxicity, biocompatibility, and controlled drug delivery to the targeted region in arthritic animal models [17].

Therefore, the current study aims to determine the effects of SeNPs on the growth rate and spleen integrity of healthy and arthritic mice to develop a new method for evaluating the efficacy of Se sources.

2. Materials and methods

2.1. Biosynthesis of SeNPs

Recently synthesized and characterized, *Trachyspermum ammi* derived biogenic SeNPs by [18] were used in this study.

2.2. Compliance with ethics guidelines

The Institutional Review Board (IRB), Atta-ur-Rahman School of Applied Biosciences (ASAB) approved procedures and protocols were used in this study (Reference Number: IRB-118). The experimentation was done according to the guidelines and recommendations of National Institute of Health's (NIH) for animal research and analysis.

2.3. Acquisition of animals

8-12 weeks old female Balb/c mice of about 30-35 grams body weight obtained from Animal House facility ASAB were used in this study. All mice were housed in plastic cages at the Laboratory of Animal House, ASAB with controlled daylight to darkness ratio (2:1) and were fed with UV-radiation treated water and standard feed throughout the study.

2.4. Toxicity evaluation of SeNPs on spleen and growth of healthy mice

For biosafety evaluation of biogenic SeNPs, their toxicity was checked in 25 healthy mice, randomly divided into five groups i.e., four experimental groups and one healthy control. Four doses of SeNPs (2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 20 mg/kg) were given to experimental groups

through feed for 14 days consecutively while the control group was fed with the normal feed during this period. The tail length of each mice was recorded with the help of (GmBH) vernier caliper from day 0-14 of treatment on weekly basis and the weight of all mice was also noted on same days to observe the effect of SeNPs on the growth of mice. At the end of treatment, the mice were dissected after overnight fasting and the spleen tissue was collected to observe the effect of SeNPs on spleen growth. The spleen weight of each mice was recorded with the help of electric weighing balance while its length was measured by using (GmBH) vernier caliper.

2.5. Arthritic model construction and effects of SeNPs on their spleen and growth

Bovine Type-II Collagen (Worthington Biochemical corporation, Lakewood, New Jersey, United States) and Complete Freund's adjuvant (Sigma-Aldrich, St. Louis, Missouri, USA) was used to induce RA by using the protocol described by [18]. After 7 days of arthritis induction, therapeutic potential of SeNPs was evaluated in 20 mice divided into four groups; one control (RA), Leflunomide (10 mg/kg) a standard drug, and two doses of SeNPs (5 mg/kg and 10 mg/kg) selected by biosafety profiling were fed to arthritic mice for 14 days consecutively while normal feed was given to control group in this period.

The weight of all mice was also noted from day 0-14 of treatment on weekly basis to observe the effect of SeNPs on the growth of arthritic mice. At the end of treatment, the spleen tissue was collected by above mentioned procedure after overnight fasting to observe the effect of SeNPs on spleen growth. The length and weight of spleen was recorded by the aforementioned procedure while the spleen indexing was performed by using the procedure previously described by [19, 20].

2.6. Statistical analysis

The statistical analysis was performed by using GraphPad Prism 5 and IBM SPSS version 21 and the results were presented as mean ± SD. The variance analysis amongst the groups was evaluated by applying one-way ANOVA along with Turkey's test and two-way ANOVA followed by Bonferroni's test. The variation at $P < 0.05$, $P < 0.01$ and $P < 0.001$ was considered statistically significant.

3. Results and discussion

3.1. Effects of SeNPs on the spleen and growth of healthy mice

Various recent studies focused on Se due to minor difference between the toxic and therapeutic dose as both the excess and deficiency of Se are harmful [21]. The present results showed significant variation in the growth rate among different doses of SeNPs as a group receiving 2.5 mg/kg SeNPs showed a significant decrease ($P < 0.05$) in the growth and the 5 and 10 mg/kg groups showed no significant reduction as compared to the control group in accordance to the results of [7, 21]. While the group receiving 20 mg/kg SeNPs significantly increases ($P < 0.001$) the growth rate of mice (Figure-1) opposite to the finding of [4]. The tail length was also found significantly increased ($P < 0.001$) after day-7 in the groups receiving SeNPs at concentration of 5, 10, and 20 mg/kg as shown in (Figure-2) comparable to the findings of [7, 22].

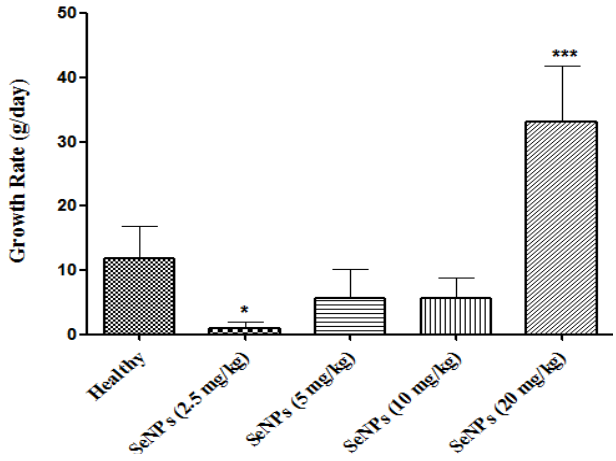

Fig. 1. Graph represents the growth rate of SeNPs treated healthy mice at concentrations of (2.5, 5, 10, and 20 mg/kg) in comparison to untreated healthy mice. SeNPs 5mg/kg showed a significant decrease in the growth of mice (P<0.05) indicated by (*) in graph, while 20 mg/kg SeNPs showed significant increase (P<0.001) (***) with respect to the control group. Statistical analysis was performed using one-way ANOVA followed by Turkey's t-test post hoc analysis and results are reported as mean ± SD (n=5 mice for all the groups) (R2=0.8667).

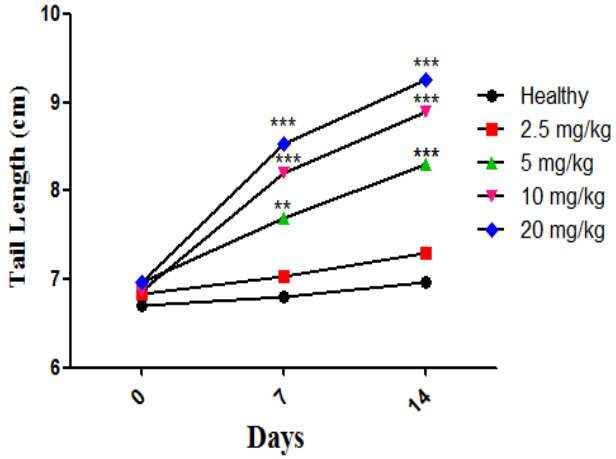

Fig. 2. Tail length of SeNPs-treated healthy mice at day 0, 7, and 14 of treatment. Statistical analysis was performed using two-way ANOVA followed by Bonferroni's post hoc analysis and results are reported as mean ± SD.

It is revealed by recent studies that Se has an important role in regulation of stress, erythroid progenitors, and spleen microenvironment and is very important for the function and development of spleen (12). To assess the toxic effects of SeNPs on spleen, its length and weight were measured, and no significant difference was found in the spleen weight among the therapeutic groups (Figure-3) as compared to healthy control while the increase in spleen weight was reported by [21]. The splenomegaly was found in the groups receiving 2.5, 5, and 20 mg/kg SeNPs (Figure-4) comparable to the results of [21] while the [7] reported the decrease in the spleen length.

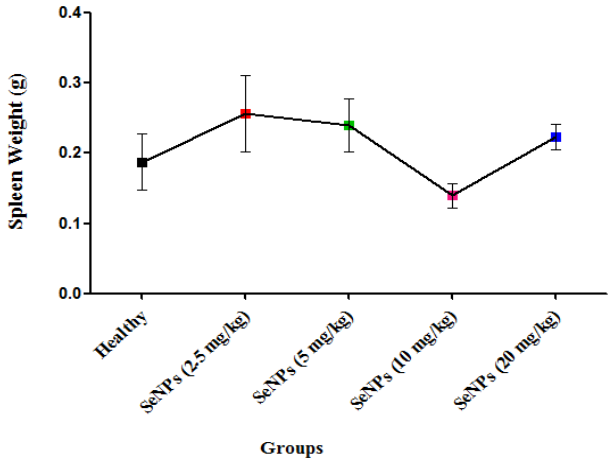

Fig. 3. Spleen weight of SeNPs treated healthy mice in comparison to the untreated healthy control. Statistical analysis was performed using one-way ANOVA followed by Turkey's t-test post hoc analysis and no significant difference was found in the spleen weight of the treated groups with respect to the untreated control (P=0.2779).

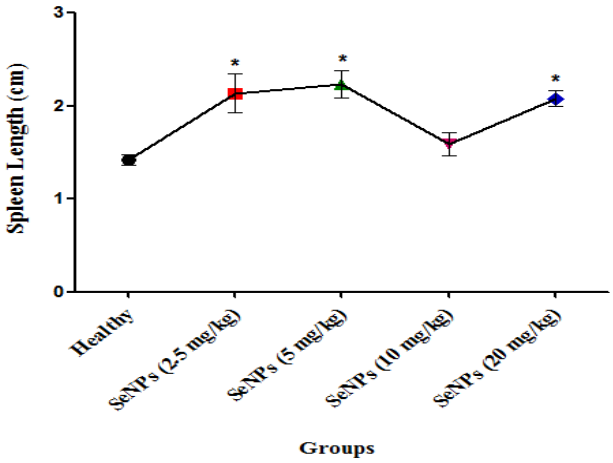

Fig. 4. Spleen length of SeNPs treated healthy mice in comparison to the untreated healthy control. Statistical analysis was performed using one-way ANOVA followed by Turkey's t-test post hoc analysis and a significant increase in the spleen length was observed in 2.5, 5, and 20 mg/kg treated mice with respect to the untreated control (P=0.0055).

3.2. Effects of SeNPs on the spleen and growth of arthritic mice

Nano-selenium due to its anti-oxidant potential can be used as chemo-therapeutic and anti-oxidant agent with low toxicity [9, 23]. At present, the therapeutic efficacy of biogenic SeNPs was elucidated by administration of Leflunomide, a standard drug at concentration of 10 mg/kg along with two doses of SeNPs (5 mg/kg and 10 mg/kg) to arthritic mice through feed. Approximately 5–10 % [24] RA patients presented splenomegaly [24-26] and they have low levels of selenium than the normal individuals [8] so to assess the therapeutic effects of SeNPs on spleen we evaluated spleen length, weight, and indices in arthritic Balb/c mice.

Selenium nanoparticles treated mice showed slight increase in the growth of arthritic mice but a significant increase ($P < 0.001$) in the growth of Leflunomide treated group was observed as compared to untreated arthritic control group (Table-1). And no splenomegaly was observed as no significant difference was found in length, weight, (Table-1) and indices of spleen among the treated and untreated arthritic mice showing that biogenic SeNPs didn't have any harmful effects on spleen (Figure-5) similar to the results reported by [27].

Table 1. Spleen and growth parameters of SeNPs and leflunomide treated groups in comparison to untreated arthritic mice (therapeutic evaluation).

Variables	Groups			
	RA	Leflunomide	SeNPs (5 mg/kg)	SeNPs (10 mg/kg)
SGR (g/day)	2.62 ± 1.8	10.71 ± 1.9***	4.28 ± 1.9	4.99 ± 3.3
Spleen Weight (g)	0.24 ± 0.05	0.18 ± 0.04	0.23 ± 0.03	0.25 ± 0.03
Spleen Length (cm)	1.74 ± 0.2	1.79 ± 0.1	2.03 ± 0.2	2.30 ± 0.2

Notes: *** Significant difference in the growth rate of leflunomide treated-arthritic group ($P < 0.001$) was found in comparison to the untreated-arthritic control group.
Abbreviations: RA, Rheumatoid Arthritis; mg/kg, milligrams per kilogram; SeNPs, Selenium Nanoparticles; SGR, Standard Growth Rate; g, Grams; cm, Centimeter.

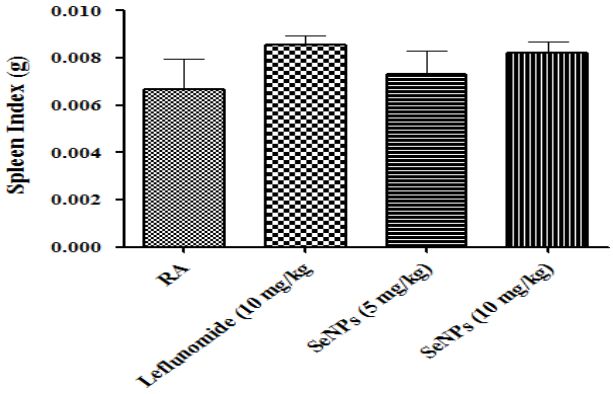

Fig. 5. Spleen indices of Leflunomide and SeNPs treated arthritic mice in comparison to the untreated arthritic control group. Statistical analysis was performed using one-way ANOVA followed by Turkey's t-test post hoc analysis and no significant difference was found in the spleen indices of the treated groups with respect to the untreated control (P = 0.1012).

4. Conclusion

The results of current study (Figure-6) provide an important information about the effect of SeNPs on the spleen indices and growth of healthy and arthritic Balb/c mice.

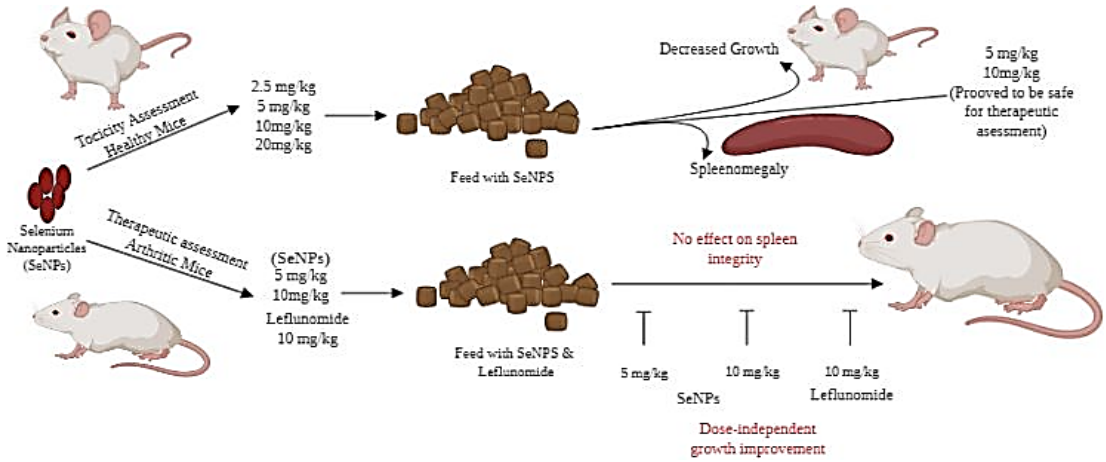

Fig. 6. Brief Summary of results.

Concluding, in biosafety profiling, the growth of healthy mice was decreased along with splenomegaly by SeNPs administration confirming Se intoxication. While the growth of arthritic

mice was increased by SeNPs treatment with no change in the spleen length indicating its therapeutic potential for arthritic mice.

References

[1] S. S. Kathiervelu, Applications of nanotechnology in fiber finishing. Synth. Fibres 32, 20 (2003).

[2] J. Kasthuri, S. Veerapandian, N. Rajendiran, Colloids and Surfaces B: Biointerfaces 68(1), 55 (2009); https://doi.org/10.1016/j.colsurfb.2008.09.021

[3] M. A. Franco-Molina, E. Mendoza-Gamboa, C.A. Sierra-Rivera, R. A. Gómez-Flores, P. Zapata-Benavides, P. Castillo-Tello, J.M. Alcocer-Gonzalez, D.F. Miranda- Hernandez, R.S. Tamez-Guerra, C: Rodríguez-Padilla, Journal of Experimental & Clinical Cancer Research, 29(1), 1 (2010); https://doi.org/10.1186/1756-9966-29-148

[4] G. Rajakumar, T. Gomathi, M Thiruvengadam, V. D. Rajeswari, V. N. Kalpana, I. M. Chung, Microbial pathogenesis, 103, 123 (2017); https://doi.org/10.1016/j.micpath.2016.12.019

[5] Y. Altav, A. L. Baş, F. Erci, E. Kocabaş,. Dicle Üniversitesi Veteriner Fakültesi Dergisi, 12(2), 149 (2019).

[6] C. F. Xie, H. X. Lou, Chemistry and Biodiversity. 6(3), 303 (2009); https://doi.org/10.1002/cbdv.200700450

[7] Ö. Yayıntas, L. Irkın, Journal of Scientific Perspectives, 2(1), 71 (2018); https://doi.org/10.26900/jsp.2018.07

[8] B. Aslanbaba, S. Yılmaz, Ö. Yayıntaş, D. Özyurt, B. D. Öztürk, Journal of Scientific Perspectives. 1(1), 1 (2017); https://doi.org/10.26900/jsp.2017.0

[9] D. Onbasli, G. Celik, E. M. Altuner, B. Aslim, Int. Pharm. Nat. Med. 7(1), 10 (2019).

[10] M. Veljic, A. Duric, M. Sokovic, A. Ciic, J. Glamoclija, P. D. Marin, Archives of Biological Sciences, 61(2): 225 (2009); https://doi.org/10.2298/ABS0902225V

[11] Ö. Yayintas, S. Yilmaz, M. Sökmen, Indian Journal of Traditional Knowledge, 18(2), 395 (2019).

[12] Ö. Yayintas, N. Demir, Fresenius Environmental Bulletin, 30(4), 3420 (2021).

[13] T. M. Lunic, M. R. Mandic, M. M. Oalde Pavlovic, A. D. Sabovljevic, M. S. Sabovljevic, B. D. Nedeljkovic, B. D. Bozic, Plants, 11(1), 123 (2022); https://doi.org/10.3390/plants11010123

[14] E. M. Altuner, K. Canli, I. Akata, Journal of Pure and Applied Microbiology, 8(1), 539 (2014).

[15] B. Dulger, Ö. Yayintas, A. Gonuz, Fitoterapia, 76, 730 (2005); https://doi.org/10.1016/j.fitote.2005.07.006

[16] CLSI, (2006), Clinical and Laboratory Standarts Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard-Seventh Edition, M07- A7, Villanova, PA, USA.

[17] M. S. Blois, Nature 181, 1199 (1958); https://doi.org/10.1038/1811199a0

[18] X. Qiao, Z. Y. Ma, C. Z. Xie, F. Xue, Y. W. Zhang, J. Y. Xu, Z. Y. Qiang, J. S. Lou, G. J. Chen, S. P. Yan, J. Inorg. Biochem. 105, 728 (2011); https://doi.org/10.1016/j.jinorgbio.2011.01.004

[19] D. M. Maron, B. N. Ames, Mutation Research/Environmental Mutagenesis and Related Subjects 113, 173 (1983); https://doi.org/10.1016/0165-1161(83)90010-9

[20] K. Mortelmans, E. Zeiger, Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 455, 29 (2000); https://doi.org/10.1016/S0027-5107(00)00064-6

[21] R. Sharma, M. Mishra, B. Gupta, C. Parsania, S. L. Singla-Pareek, A. Pareek, De PloS one, 10(5), e0126783 (2015); https://doi.org/10.1371/journal.pone.0126783

[22] Y. F. Xian, Z. Hu, S. P. Ip, J. N. Chen, Z. R. Su, X. P. Lai, Z. X. Lin, Phytomedicine, 50, 196 (2018); https://doi.org/10.1016/j.phymed.2018.05.010

[23] A. J. Vieira, F. P. Beserra, M. C. Souza, B. M. Totti, A. L. Rozza, Chemico-Biological Interactions, 283, 97 (2018); https://doi.org/10.1016/j.cbi.2018.02.007

[24] A. Hatipoğlu, Gümüş nanopartiküllerin yeşil sentezi ve bazı gıda patojenleri üzerindeki antimikrobiyal etkileri, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 26, 1 (2022); https://doi.org/10.19113/sdufenbed.970654

[25] M. F. Baran, A. Saydut, U Adil, Gümüş nanomalzeme sentezi ve antimikrobiyal uygulamaları, Dicle Üniversitesi Mühendislik Fakültesi Mühendislik Dergisi, 10(2), 689 (2019); https://doi.org/10.24012/dumf.518897

[26] A. Eren, M. F. Baran, Fıstık (Pistacia vera L.) yaprağından gümüş nanopartikül (AgNP)'lerin sentezi, karakterizasyonu ve antimikrobiyal aktivitesinin incelenmesi, Türkiye Tarımsal Araştırmalar Dergisi, 6(2), 165 (2019); https://doi.org/10.19159/tutad.493006

[27] Ö. Yayintas, N. Demir, S. Yılmaz, M. M. Çiçekliyurt, Digest Journal of Nanomaterials and Biostructures, 25(4), 1487 (2021); https://doi.org/10.15251/DJNB.2021.164.1487

[28] M. Singh, S. Singh, S. Prasad, I. S. Gambhir, Digest Journal of Nanomaterials and Biostructures, 3(3), 115 (2008).

[29] P. Cianciullo, V. Maresca, S. Sorbo, A. Basile, Applied Sciences, 12(1), 160 (2022); https://doi.org/10.3390/app12010160

[30] M. Vollar, A. Gyovai, P. Szucs, I. Zupk, M. Marschall, B. Löffler, P. Berdi, A. Vecsernyes, A. L. Csorba, E. Busa, E. Urban, D. Csupor, Molecules, 23, 1520 (2018); https://doi.org/10.3390/molecules23071520

[31] S. Srivastava, A. Bhargava, Biological synthesis of nanoparticles: Bryophytes. In Green nanoparticles: The future of nanobiotechnology, Springer, Singapore, 173 (2022); https://doi.org/10.1007/978-981-16-7106-7_8

[32] S. S. Sathish, P. Vijayakanth, Ind J Plant Sci, 5, 56 (2016).

[33] Ö. Ertürk, H. Sahin, E. Y. Ertürk, H. E. Hotaman, B. Koz, Ö. Özdemir, Herba Polonica, 61(4), 52 (2015); https://doi.org/10.1515/hepo-2015-0031

[34] Ö. Yayıntaş, N. Demir, Fresenıus Environmental Bulletin, 28(9), 6978 (2019).