Original Article

In Vitro Determination of the Antibacterial Activity of AgNPs using Syzygium Aromaticum Against Streptococcus Mutans

Mushtaq Talip Hussein

ABSTRACT

Objective: To evaluate the antimicrobial activity of AgNPs with clove extract against Streptococcus mutans and determine their MIC and MBC

Study Design: Controlled, in vitro laboratory experimental study

Place and Duration of Study: This study was conducted at the Department of Basic Sciences, College of Dentistry, Al-Qadisiyah University, Qadisiyah Governorate, Iraq, from 14th Jan 2025 to 20th September 2025.

Methodology: Biosynthesis of SA AgNPs and characterization using UV-Vis, FTIR, XRD, and SEM. The antibacterial activity was determined by the agar well diffusion method then estimate the MIC and MBC.

Results: The XRD pattern of the pure silver sample has a closely match JCPDS card No. 04-0783 with four sharp, visible diffraction peaks appeared at specific 2Θ angles. FTIR spectroscopy of SA AgNPs identified the specific functional groups. High absorption of AgNPs appeared at 421nm in the UV-Vis spectrum. SEM analysis showed a crystalline nature and quasi-spherical form of AgNPs with dimensions 14 - 32 nm. The zones of inhibition for AgNPs synthesized using Syzygium aromaticum were determined using agar well diffusion method. SA AgNPs at a concentration of 20 mg/mL displayed the most potent antibacterial action, producing the largest zone of inhibition measuring 19 ± 0.8 mm. The inhibitory activity was initiated at 0.32 mg/ml, while the MBC was 1.25 mg/ml. The calculated MBC/MIC ratio was determined to be approximately 3.9 (∽4).

Conclusion: The results suggested that Ag nanoparticle synthesis with Syzygium aromaticum can be used as an effective inhibitor of bacterial growth.

Key Words: Silver, Extraction, Clove, Gram-positive, Antibacterial agents.

doi:http://doi.org/10.60110/medforum.37.10.24

Citation of article: Hussein MT. In Vitro Determination of the Antibacterial Activity of AgNPs using Syzygium Aromaticum against Streptococcus Mutans. Med Forum 2026;37(10):124-128. doi:http://doi.org/10.60110/ medforum.37.10.24.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0) (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

One of the important fields is Nanotechnology. There is an intersection between the biomedical sciences and nanotechnology and act to produce novel substances with therapeutic ability. AgNPs have antibacterial, anticancer, and catalytic applications because of their physicochemical characteristics. The synergistic action of clove extract and silver nanoparticles produces a bioactive substance with antibacterial activity.[1] Tooth caries was an important type of biofilm-mediated illness that contains several types of cariogenic.[2] Teeth caries lead to occurrence of cardiovascular diseases.[3] Oral conditions play effective roles in the accepted caries etiology.[4] Frequently, sugars intake leads to acid production, and cause unbalance between acidogenic and none-acidogenic microorganisms.[5] S.mutans was a main causes in the dental caries in 70 to 90 percent, due to it produces lactic acid as well as grows at lower pH. Caries development is depended on the virulence of mouth micro-organisms that participate in its emergence.[6] There are many studies that refer to use clove in treating dental diseases due to antibacterial effects, with fewer side effects and used as toothpaste due to its strong effects and availability and low cost. Cloves oil has two major components, eugenol and β-caryophyllene. The first one has bactericidal effects on several types of bacteria.[7]

Department of Basic Science, Faculty of Dentistry, Al-Qadisiyah University, Diwaniyah, Iraq.

Correspondence: Mushtaq Talip Hussein, Department of Basic Science, College of Dentistry, University of Al-Qadisiyah, Diwaniyah, Iraq.
Contact No: +9647812403390
Email: mushtaq171981@gmail.com

Received: June, 2026
Reviewed: July-August, 2026
Accepted: September, 2026

This study aims to evaluate the green synthesis, characterization, antimicrobial activity, and biomedical activity of SA AgNPs under optimized reaction conditions, including temperature, pH and concentrations.

METHODOLOGY

a. Clove extract preparation: Plant sample materials were collected from popular markets in Al- Diwaniyah, Iraq, and the taxonomic identification of the plant was confirmed as Syzygium aromaticum by the Department of Biology at Babylon University, Iraq. The washed plant were sterilized and dried far from light. The dried material was crushed into fine particles. A portion of the powder (2 gram) was dissolved in distilled water (100 mL) by shaking for 48h and filtered on cotton and allowed to cool to 25ºC. To ensure a clear extract, the filtrate was filtered through a Whatman No. [41] filter paper then Centrifugation was performed at 1600 rpm for 10 min. The clarified leachate was concentrated by a rotary vacuum evaporator at reduced pressure and dried at 40 °C. The sample was weighed and stored in a small container in cold conditions at 5 °C until further use.[8]

b. Green synthesis of Ag NPs: The biosynthesis of silver nanoparticles can be performed using a 1 mM solution of $AgNO_3$ and clove extract. Biosynthesis is performed by adding 5 mL of extract to 50ml of 1 mM $AgNO_3$ solution. In the next step, incubation of the mixture carried out at 90 °C for 2 min to complete the reduction process.[9] A stock solution with a concentration of 20 mg/mL was prepared as the final concentration of SA AgNps which used in the antibacterial assay.

c. Characterization of Ag nanoparticles: The methods used to characterize the purified nanoparticles:

1. UV-vis Spectroscopy: UV-vis Spectroscopy (SpectraMax 340 PC, Molecular Devices, Munich, Germany) was used to monitor the biological reduction of Ag+ to Ag0 and measure of the absorption spectra (UV-vis) over a range of 280 to 700 nm. Readings were recorded twice within 15 min.[10]

2. Infrared Spectrophotometer and XRD: An infrared spectrophotometer (Bruker Tensor 27 IR spectrometer) was carried out to identify the clove functional groups. These groups were responsible for capping and reduction of nanoparticles.[11]

To analyze the crystalline structures of the samples, an X-ray diffractometer (XRD) (Shimadzu model: XRD 6000 with CuKα radiation at 1.5406ƛ, operated at 40 kV and 30 mA) was used. Analysis of the shape of the particles was performed by a particle size analyzer (Sympatec GmbH, NANOPHOX).

3. Scanning electron microscopy: Detection of the morphology and size of AgNPs was performed by SEM. The oven-dried SA-AgNP (thin films) were made and provide for use on a carbon –coated copper grid via a TESCAN MIRA-3 instrument operated at an accelerated voltage of 5.0 kV.[11]

d. Antimicrobial activity determination:

1. Bacterial suspension: The bacteria were obtained from the culture collection department of the Micro Lab in Al-Diwanyia Hospital, Iraq. The suspension was made by choosing 3-5 pure colonies. These colonies were suspended in 5mLof sterile saline solution and adjusted to match the 0.5 McFarland

2. Antimicrobial activity of extracts: The well-diffusion method was used to determine the antimicrobial action of the clove extract. The bacterial suspension turbidity was standardized to a 0.5 McFarland. Mueller-Hinton agar was incubated with the bacterial suspension. 6 mm wells were punched into the medium and filled with 100 µL of the various concentrations (100, 50, 25 mg/ml) of Syzygium aromaticum extracts. In the negative control, wells were filled with 100 µL of distilled water. Each plate was incubated for 24 h at 37°C. The zones of inhibition were measured in mm.[12]

3. Antibacterial activities of AgNPs: Streptococcus mutans was cultured onto Mueller-Hinton agar and incubated overnight at 35 °C. A 0.5 McFarland standard was utilized to the turbidity standardization of the suspension of bacteria. The antimicrobial activities of AgNPs synthesized with clove were evaluated and compared with a positive control (Carbenicillin1mg/mL), alongside the clove extract. The agar well diffusion method was used under sterile conditions. After spread of the bacterial suspension, the wells were punched into the medium and filled with 100 µL of the respective treatment, and a negative control was included. The experiments were performed in triplicate. Finally, the MIC and MBC were estimated by microdilution broth test.[12]

4. MIC and MBC determination: The MIC and MBC of AgNPs synthesized by clove extract were determined by the broth microdilution method.[12]

e. Statistical analysis: Data are presented as mean ± standard error of the mean, and all antibacterial assays were conducted in triplicate. Statistical analysis was performed using the standard error of the mean.

RESULTS

a. Characterization of AgNPs:

1. UV-vis spectral analysis: The addition of the SA extracts to the $AgNO_3$ solution led to a distinct color change from origin color (yellow) to brown due to the Ag+ reduction to Ag nanoparticles; this visual change confirmed the synthesis of AgNPs (Figure 1). Figure 2 shows the UV–visible absorption spectra of AgNPs using a 2 mM $AgNO_3$ concentration with a plant extract after 24 hours at 130°C via the autoclave- assisted method. The absorption spectrum extended from 400 to

500 nm, with a maximum surface plasmon resonance peak observed at 421 nm.

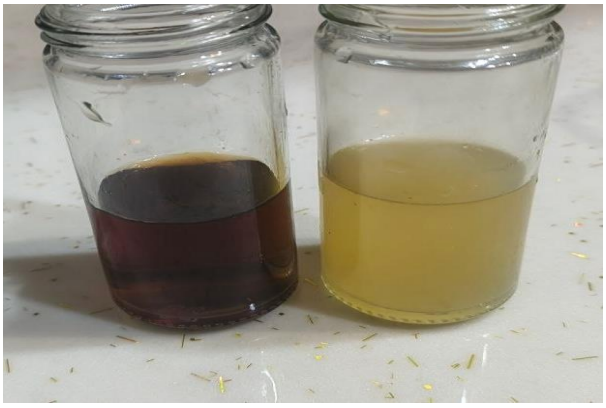


Figure No. 1. The color change of solution to brown due to the Ag+ reduction to Ag NPs.

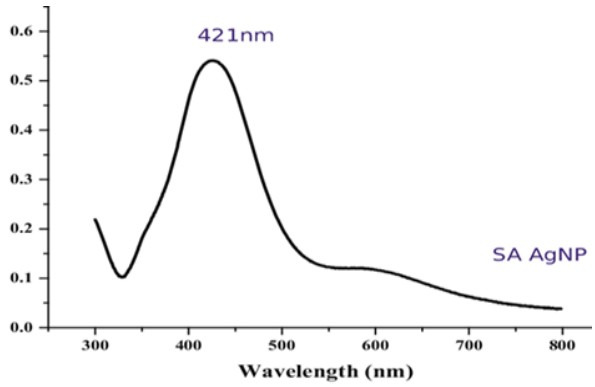


Figure No. 2. The peak AgNPs absorption appeared at 421 nm in the UV-Vis spectrum

2. Functional groups analysis: FTIR spectroscopy of silver nanoparticles with clove extract was groups responsible for reducing silver ions (Ag^{+}) into metallic silver (Ag^{0}) and stabilizing the resulting nanoparticles. Figure (3). (Table 1) utilized to characterize the specific functional groups.

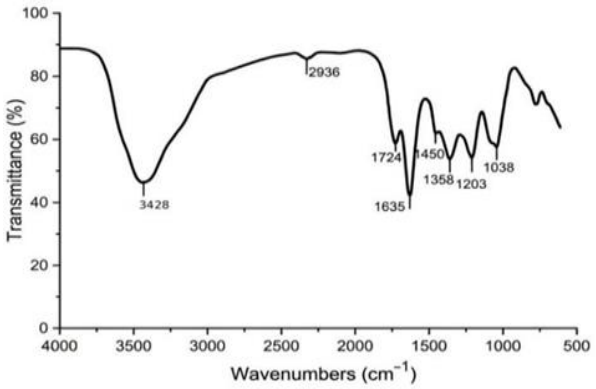


Figure No. 3. FTIR spectrum of SA AgNPs shows peaks and their corresponding functional groups.

Table No. 1. FTIR spectrum of SA AgNPs shows the biomolecules involved in capping and reduction of silver nanoparticles

Approximate Wavenumber (cm^{-1})	Functional Group Assignment	Extend bimolecular source in SA extract
3428	O-H Stretching	Phenolic compounds, flavonoids, and eugenol
2936	C-H Stretching	Aliphatic CH groups
1724	C=O Stretching	Ester group of eugenol estate
1635 → 1610	C=C Stretching	Aromatic rings or allyl group of eugenol and carbonyl groups from proteins, flavonoids, and terpenoids
1450 / 1358	C-H Bending	Organic molecules capping the nanoparticle surface
1203 / 1038	C-O Stretching	Polyols, ethers, or alcoholic groups; phenols

3. XRD characterization synthesized silver nanoparticles: The XRD pattern of the pure Ag sample shows high compatibility with JCPDS card No. 04-0783. The four sharp, distinct diffraction peaks appeared at specific 2Θ angles. These peaks correspond to the (111), (200), (220), and (311) Miller crystal planes (Figure 4). The (111) plane represents the highest intensity (Table 2).

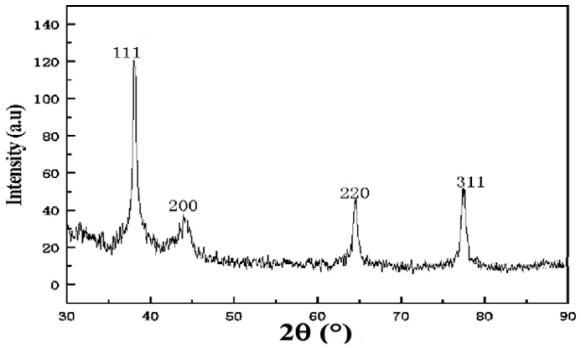


Figure No. 4. Structural characterization and phase purity of synthesized silver nanoparticles.

Table No. 2. XRD data for AgNPs nanoparticles corresponding to the standard FCC phase.

Diffraction (2Θ º) angle	Crystal plane (hkl)	Crystal plane (hkl)
38.1º	(111)	Highest intensity
44.3º	(200)	Medium intensity
64.4º	(220)	Low intensity
77.4º	(311)	Low intensity

4. Scanning Electron Microscopy (SEM): Figure 3 showed the nanoparticles with a predominantly spherical morphology with dimensions ranging from 14 to 32 nm. The observed narrow size distribution aligns well with the results of the UV-Vis spectrophotometric data (Figure 5).

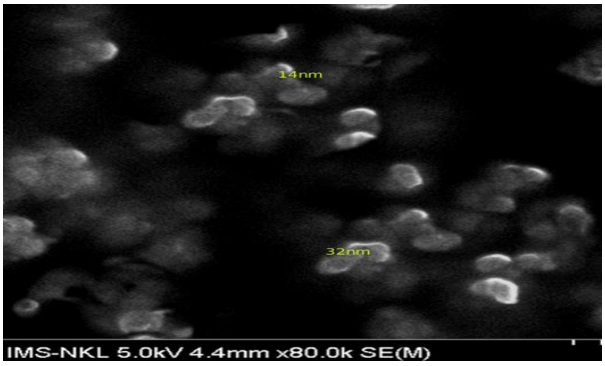


Figure No. 5. The result of SEM showed dimensions ranging from 14 to 32 nm and appeared as spherical symmetry.

f. Estimation of Antibacterial activity: Aqueous clove extract exhibited a clear concentration-dependent antimicrobial efficacy. A 25 mg/mL concentration showed a mean inhibition zone 9±0.5 mm, while 50 mg/mL and 100 mg/ml improved the antibacterial action, producing mean inhibition zones of 11 ± 0.57 mm and 15 ± 1.3 mm, respectively. SA AgNPs at a concentration of 2.5 mg/ml displayed the most potent antibacterial action among all tested materials, producing the largest zone of inhibition measuring 19 ± 0.8 mm. Carbenicillin (1 mg/ml), the positive control, generated a zone of inhibition of 16 ± 0.8 mm, which was lower than the SA AgNPs. In contrast, sterile water (the negative control) showed no inhibitory activity (0 mm) against the tested bacteria - Table (3).

Table No. 3. In vitro antimicrobial effect of aqueous, SA Ag NPs and Carbenicillin against S. mutans

Treatment material	Concentrations	Loading quantity per well	Inhibition zones (mm) (mean ±SR)
Inhibition zones (mm) (mean ±SR)	25mg/mL	100µ	9±0.5
	50mg/mL	100µl	11±0.57
	100mg/mL	100µl	15±1.3
SA AgNPs	20 mg/mL	100µl	19±0.8
Carbenicillin (positive control)	1mg/mL	100µl	16±0.8
Water (negative control)	-	100µl	0

Data are expressed as means of three replicates (n=3) ±standard error.

g. Determination of MIC and MBC of SA AgNPs: Determination of the MIC and MBC of AgNPs were done by the broth microdilution method. The antibacterial activity of the AgNPs with clove extract was recorded in Table (4). The inhibitory activity was initiated at 0.32 mg/ml whereas MBC ranged from 1.25 mg/ml. The calculated MBC/MIC ratio was determined to be approximately 3.9 (∽4).

Table No. 4. MIC and MBC of SA AgNPs

AgNPs	Conc. mg/ml	MBC/MIC Ratio	Antibacterial action
	0.16	3.9(≈4)	Bactericidal
MIC	0.32		
	0.64		
MBC	1.25		
	2.5		
	5		
	10		

DISCUSSION

The current study aimed to synthesize nanoparticles with antibacterial effects using Syzygium aromaticum extract. Several studies have mentioned the microbicidal activity of SA Ag NPs. So, this study was similar to that reported by Alharth et al.[13]

After 24 hours, the result exhibited a brown color, indicating the formation of silver nanoparticles in solution. This aligns with the previous study of Palanivelu et al.[14] The in vitro antibacterial activity results exhibited varying diameters of inhibition zones. There is a direct relationship between the inhibition zone diameter and the concentration of the clove extract due to density of bioactive materials, particularly eugenol - Mansoori et al.[15] Numerous documents indicate the antimicrobial properties of both silver nanoparticles (AgNPs) with clove extract. This potent antimicrobial activity is closely proportionate with recent results of Molina-Trinidad et al[16] and other studies of AgNPs with clove extract, which reported inhibition zones ranging from 18 mm to 20 mm.[17] Devi and Bhaskar demonstrate the eugenol-functionalized nanoparticles give lower Minimum Inhibitory Concentration (MIC) due to cooperating action against oral streptococci.[18] This study findings showed intrinsic differences when compared to recent literature. Our result disagreed with the findings of the study of Elchaghaby et al[19] showed the antimicrobial activity of Ag NPs with star anise extract against S. mutans with a lower MIC value of 0.08 mg/ml (80 µg/ml), referring to a higher bacterial sensitivity. These differences could be related to variations in bioactive phytochemical materials. Furthermore, our study differed from the study of Rajendran and Rajeshkumar, that showed a clear indicator for AgNPs synthesized using cinnamon with 10 mm inhibition zone[20].

CONCLUSION

It was concluded that S.mutans infection can treat by small size nanomaterials of Ag with a clove extract, with minimal side effects on the body.

Acknowledgments: The author is thankful to Dr. Ziad Metaab, Managing Director, Department of Biology, College of Science, Al-Qadisiyah University, for their help. The author would also like to thank Zoonosis diseases unite of the Veterinary Medicine College, Al-Qadisiyah University, for organizational assistance.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mushtaq Talip Hussein
Drafting or Revising Critically:	Mushtaq Talip Hussein
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 8795 Dated 14.01.2025

REFERENCES

1. Elhalik A, Abdel Ghany Elrahmany A, et al. Clove-mediated green synthesis of silver nanoparticles and in vitro investigation of their antimicrobial, antioxidant, and anticancer potential. Front Microbiol 2026,28;17:1860045. doi:10.3389/fmicb.2026.1860045.
2. Pitts NB, Zero DT, Marsh PD, Ekstrand K, et al. Dental caries. Nat Rev Dis Primers 2017;3:1–16. doi: 10.1038/nrdp..30.
3. Ono Y, Chou YC, Chien WC, Sun CA. Association between severity of dental caries and the risk of stroke. Oral Dis 2023;30(5):3413–21. 10.1111/odi.14756.
4. Manji F, Dahlen G, Fejerskov O. Caries and Periodontitis: contesting the conventional wisdom on their aetiology. Caries Res 2018;52:548–564. DOI: 10.1159/000488948.
5. Katrak C, Reed S, Carter M, Khatib M, et al. Oral hygiene agents at work: effects on Streptococcus mutans and caries risk. Front Cell Infect Microbiol 2026;16:1768512. doi:10.3389/fcimb.2026.1768512
6. Yanke Fang, Xin Chen, Chun Hung Chu et al. Roles of Streptococcus mutans in human health: beyond dental caries. Front Microbiol 2024; 15:1503657. doi: 10.3389/fmicb.2024.1503657.
7. Tariq H, Alhudhaibi AM, Abdallah EM. Syzygium aromaticum (clove buds) as a natural antibacterial agent: a promising alternative to combat multidrug-resistant bacteria. Front Microbiol 2025;16: 1674590. doi: 10.3389/fmicb.2025.1674590
8. Al-Thobity AM, Khan SQ. Preparation of clove extract-loaded nano-hydroxyapatite for combating oral pathogens. Front Bioeng Biotechnol 2026;14: 1412827. doi: 10.3389/fbioe.2026.1412827.
9. Oves M, Alsamman AM, Tariq H. Clove-mediated green synthesis of silver nanoparticles and in vitro evaluation of their antibacterial activity. Front Microbiol 2026;28;17:1860045. doi:10.3389/fmicb.2026.1860045.
10. Subramanian A, Ramasamy K. Green Synthesis and Characterization of Ag Nanoparticles Using Plant Extract: Evaluation of UV-Vis, FTIR, and XRD Profiles. Microsc Res Tech 2025;88(3):412-421. doi: 10.1002/jemt.24817.
11. Tavukcuoglu O, Ciftci F, Evcimen et al, AA. Green-Synthesized Silver Nanoparticles from Zingiber officinale: Physicochemical Characterization, Antibacterial Activity, and TMPRSS2-Modulating Potential. Nanomaterials 2026;16(14):836. doi: 10.3390/nano16140836.
12. Amal S, Ahmed M. Effects of clove (Syzygium aromaticum) extract on antibacterial activity, phytochemical properties, and storage quality of functional dairy products. J Dairy Sci 2025; 108(4):2841-2852. doi: 10.3168/jds.2024-26023.
13. Alharthi S, Al-Zharani M, Alharbi O, et al. Silver Nanoparticles in Antibacterial Research: Mechanisms, Applications, and Future Perspectives. Int J Mol Sci 2026;27(2):927. doi: 10.3390/ijms27020927.
14. Palanivelu J, Kunjumon MM, et al. Green synthesis of silver nanoparticles from Dracaena mahatma leaf extract and its antimicrobial activity. J Pharm Sci Res 2015;7: 690–695.
15. Al-Mansoori D, Al-Utbi AW, Al-Muana AM. Green synthesis of silver nanoparticles using Syzygium aromaticum (clove) extract and evaluation of their antibacterial and antibiofilm activity against oral pathogens. Asian J Agric Biol 2026;2026(1):1-10. Available from: doi.org DOI: 10.35495/ajab.2026.01.055.
16. Molina-Trinidad EM, Orozco-Cisneros RE, et al. Antimicrobial Activity of Biogenic Silver Nanoparticles from Syzygium aromaticum Against Oral Microorganisms. Antibiotics (Basel) 2022; 11(7):834. DOI: 10.3390/antibiotics11070834.
17. Mesa-Espinoza K, Flores-Coronado M, Solís-Ruiz C, et al. Antimicrobial Activity of Biogenic Silver Nanoparticles from Syzygium aromaticum against the Five Most Common Microorganisms in the Oral Cavity. Antibiotics 2022;11(7):834. doi:10.3390/antibiotics110708342) .
18. Devi S, Bhaskar R. Eugenol-functionalized nanoparticles with enhanced antimicrobial activity and lower minimum inhibitory concentration against oral streptococci. J Oral Microbiol 2022; 14(1):2054321. doi:10.1080/20002297.2022.2.
19. Elchaghaby MA, Rashad S, Wassef NM. Bioactivity and antibacterial effect of star anise biosynthesized silver nanoparticles against Streptococcus mutans: an in vitro study. BMC Complementary Medicine and Therapies 2024; 24(1):259. Available from: Springer Nature / BMC Journal. DOI: 10.1186/s12906-024-04550-x.
20. Rajendran S, Rajeshkumar S. Antibacterial efficacy of Cinnamon-Clove mediated Silver nanoparticles against Streptococcus mutans. Plant Cell Biotechnol Molecular Biol 2020;21(39-40):113-119. DOI: 10.56557/pcbmb/2020/v21i39-405348.