Fayoum Journal of Agricultural Research and Development
ISSN:1110-7790
ONLINE ISSN:2805-2528

In-Vitro antiviral activity of chitosan nanoparticles against Banana bunchy top virus

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ABSTRACT

Banana bunchy top virus (BBTV) is considered as a destructive viral disease affecting banana plants in Egypt. Recently, nanotechnology approach was used to generate resistance against plant viruses. The main objective of this study was to evaluate the antiviral capabilities of chitosan nanoparticles to control BBTV in banana plants in *vitro* cultured. The naturally infected banana plants were collected from open fields at different locations in Egypt. Polymerase chain reaction (PCR) was successfully amplified approximately of 747 bp using specific primers for coat protein gene. In-*vitro*, three different concentrations of Chi-NPs (50, 100, and 200 mg/l) were evaluated. The results showed that infected explants with BBTV were symptomless, the enzyme-linked immunosorbent assay (ELISA) produced negative results. Biochemical analysis in infected explants showed a significant increase in polyphenol oxidase and peroxidase enzymes as well as a significant increase in total oxidant activity in compression with healthy ones. Infected explants treated with Chi-NPs showed a significant increase in total phenols, total soluble sugar, protein, and malondialdehyde, compared to those of untreated infected control. Collectively, we can conclude that Chi-NPs are a potential antiviral remedy against BBTV and could be considered in virus management.

Keywords: Banana bunchy top virus, PCR, serological detection, nano materials, tissue culture.

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Submit date: 07-11-2024
Revise date: 09-01-2025
Accept date: 09-01-2025

1. INTRODUCTION

Bananas and plantains (family *Musaceae*) are tropical fruit crops that are lucrative food crops that can be used as cash crops for local markets and exports, especially in the developing nations, or as staple foods in some countries. After rice, wheat, and milk, bananas are regarded as the fourth most important food in the world **(Rahayuniati *et al.*, 2021)**. Banana bunchy top disease (BBTD) has been considered one of the serious biotic and economically important diseases of banana and plantain production worldwide **(Jekayinoluwa *et al.*, 2020)**. A number of viruses had been recorded on bananas in Africa, including *banana bunch top virus* (BBTV) genus *Nanovirus*, *banana streak virus* (BSV) genus *badnavirus*, *cucumber mosaic virus* (CMV) genus *cucumovirus*, *banana dieback virus* (BDBV) genus *nepovirus*, *banana mild mosaic virus* (BanMMV) family *Flexiviridae*, and *banana bract mosaic virus* (BBMV) genus *Potyvirus* **(Kubiriba and Tushemereirwe, 2003)**. *Banana bunchy top virus* consists of 6 components of ssDNA, each size ranging from 1018 to 1111 bp. They are DNA-R, DNA-S, DNA-M, DNA-N, DNA-C, and DNA-U3 **(Rahayuniati *et al.*, 2021)**. *Banana bunchy top virus* (genus *Babuvirus*, family *Nanoviridae*) is a virus with isometric virions 18-20 nm in diameter that attacks the phloem tissues of banana leaves and occurs symptoms such as leaf chlorosis, vein clearing, leaf dwarfing **(Hapsari *et al.*, 2023)**. The BBTV is transmitted by the banana aphid *Pentalonia nigronervosa* Coquerel. Its transmission is affected by temperature **(Tchatchambe *et al.*, 2020)**.

Aside from its usage as a research tool, tissue culture technology is frequently used for large-scale plant multiplication. In recent years, plant tissue culture techniques have gained significant industrial significance in the multiplication of plants. Elimination of disease, enhancement of plants, and generation of secondary metabolites (**Hussain *et al.*, 2012)**. One of the strategies for disease management is using nano particles in agriculture without any environmental damage or cause an imbalance in the existing biota **(Hamid *et al.*, 2022)**. At present, there are reports of positive impacts associated with the use of nanoparticles, including antibacterial, antifungal, and antiviral properties, observed in both animals and plants. The prospective antiviral capabilities of metal nanoparticles (Me-NPs) position them as a strong alternative for managing these histological agents **(Vargas *et al.*, 2020)**. The use of chitosan may inhibit the growth and sporulation of microbial pathogens by damaging their cellular membranes and interfering with several biochemical processes involved in the interaction between the plant and the pathogen, leading to specific defensive reactions in the host plants **(Chirkov, 2002; Davydova *et al.*, 2011; He *et al.*, 2021)**. This study aims to use Chi-NPs as antiviral against BBTV *in vitro* cultures.

2. MATERIAL AND METHODS

2.1. Field observations and samples collection

Samples were collected from banana open fields at different locations in Egypt, mainly Badr area, Behira governorate. Typical symptoms of BBTD were observed in visited locations. Symptoms include severe stunting, minor veins on the leaf, and dark green streaks on the petiole.

2.2. Molecular detection

Total DNA was extracted using the I-genomic plant DNA Extraction Mini Kit according to the manufacturer's instructions. The virus was detected by PCR technique which carried out as following, dropped a12.5µL master mix in a bottom of the PCR tube then added 1µL of forward and 1µL of reverse specific primer for BBTV **(Table 1)**. on the side of the PCR tube. So that could see the drops of the primer on the side of the PCR tube. Followed by the primer addition, added 8.5µL of water into the tube which takes primer drops with it, into the bottom of the tube. Finally, at last, added 2µL of template DNA. Adding DNA, at last, minimize the chance of starting the reaction early before the PCR. This pattern of reaction always gives excellent results without any failure. The amplification reaction was automated in a T-Gradient thermal cycler (T_{100} Bio-Rad) with an initial denaturation at 95℃ for 3 min, followed by 35 cycles of denaturation at 95 ℃ for 30 sec, annealing at 56 ℃ for 45 sec and extension at 72 ℃ for 1 min, with a final additional extension step for 7 min at 72 ℃. The PCR products were electrophoresed on 1% agarose gel in 1x TBE buffer, then visualized by an UV Trans illuminator.

Table 1. Primers for PCR detection of BBTV

Primers	Sequence	Position	Amplified DNA (bp)
Forward	ATGTGGTATGCTGGATGTTC	DNA-1:139-158	747 bp
Reverse	GTTCATATTTCCCGCTTTGA	DNA-1:885-866	

2.3. Plant material and sample processing

In-vitro cultured shoot tip explants which used in this experiment were taken from suckers of naturally infected mother Grandnan banana plants with Banana bunchy top virus (BBTV). All suckers were collected from Badr Center, Behira Governorate. All samples were transferred and storage at the Tissue Culture lab, Virus and Phytoplasma Department, Plant Pathology institute. Agric. Res., Giza Center.

Suckers were placed in 70% ethanol for three min, and then washed three times, followed by 0.1% $HgCl_2$, Mercuric chloride (MC) solution with gently shaking for half hour, then washed with sterilized distilled water three times for three min for one time. Then the explanted were treated with 60% commercial Clorox solution mixed with a few drops of Tween 20 for half hour, washed three times with sterile distilled water (SDW) every three min. then were used to initiate shoot culture **Fig. 1 (Alam *et al.*, 2004)**.

2.4. Nutrient medium

Procedure for micropropagation of banana plantlets *in-vitro* cultures of infected plants was cultivated on standard media with mineral salts (**Murashige and Skoog, 1962)**. This medium was enriched with 30 g/l of

sucrose, 4.4 g/l of MS, 5 g/l Benzyl Adenine, 7 g/l agar. The PH of this medium is (5.8), to make sure that the prepared medium was not contaminated; it was kept at the storage room for 7 days before cultivation.

2.5. Sub-cultures

Following the initiation of shoot production by the explants, the initial subculture was executed. Cut into tiny pieces, each containing a single shoot, *in-vitro* shoots were prepared for subculturing. To reveal the meristem, the leaf and brownish or black basal tissues were cut away. Every piece was injected into a comparable new medium shown in **Fig. 1** according to **Shiraigi *et al*. (2008)**. The process of subculturing resulted in an increase in the number of shoots. Each subculture taking place took four weeks.

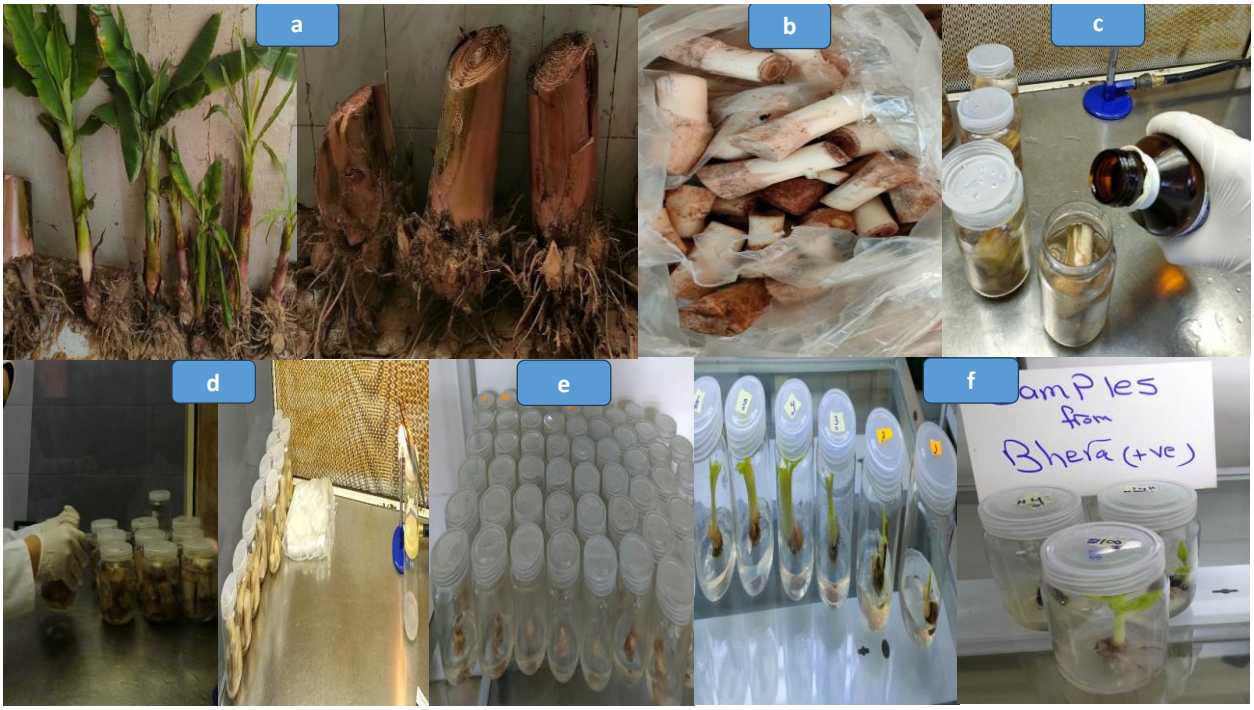

Fig. 1. The procedure for propagation of banana plantlets in *vitro* cultures of infected plants). (a) Infected samples. (b) Cutting the corms. (c and d) sample processing. (e) Planting on MS medium. (f) Sub-cultures after 4 weeks.

2.6. Chitosan preparation and characterization

Chitosan nanoparticles were prepared in the center of nanotechnology and advanced materials, at Agricultural Research Center, Giza, Egypt. (TEM) transmission electron microscope was used for morphology characterization of Chi-NPs. For Surface structure Using a 200 kV accelerating voltage, a high-resolution transmission electron microscope (Tecnai G2, FEI, Netherlands) was used to determine the particle size and the true shape of Chi-NPs. (**Loutfy *et al.*, 2022)**.

2.7. Nanotherapy treatment by chitosan nanoparticles

Following four subcultures, 5-centimeter shoots were collected from 4

weeks old stock cultures and inoculated onto MS- standardized medium. The medium was supplemented with varying concentrations (50, 100, and 200 mg/l) of Chi-NPs to evaluate its impact on the shoots' regeneration response, plantlet growth, and the elimination of BBTV.

2.8. BBTV detection after treatments by chitosan nanoparticles

After 4 weeks of the treatments, leaves were collected from the treated samples and tested for BBTV using double antibody sandwich ELISA (DAS-ELISA). The procedure of the DAS-ELISA assay was used for detecting BBTV according to the procedure described by **Clark and Adams (1977)**.

2.9. Determination of phytochemicals in BBTV infected-plants after treatments

2.9.1. Estimation of total carbohydrate

Determination of carbohydrates by anthrone sulphuric acid method **(Fales, 1951; Schlegel, 1956)**.

2.9.2. Estimation of protein

The soluble protein was then calculated using the method according to. **(Lowry *et al.*, 1951)**.

2.9.3. Determination of phenolic

From desiccated tissues, phenolic chemicals were extracted using a method which carried out by **Sauvesty *et al*. (1992)**.

2.9.4. Determination of malondialdehyde

One of the common techniques for estimating MDA, is the thiobarbituric acid-reactive-substances (TBARS) assay **(Hodges *et al*., 1999)**.

2.9.5. Determination of antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging technique is used to determine antioxidant activity, according to **Abe *et al*. (1998)**.

2.10. Determination of photopigments

Extraction and quantification of chlorophyll *a*, *b*, and carotenoids as a following procedure: fresh leaf samples (0.5 g) were homogenized in a mortar with 85% acetone, washed dried sand, and a small amount of $CaCO_3$ (0.1 g) to neutralize organic acids in the fresh leaf homogenate. After that, the homogenate was filtered through a sintered glass funnel. The residue was washed with acetone numerous times until the filtrate was colorless. A spectrophotometer was used to determine the optical density of this extract at 663nm and 645 nm for chlorophyll a and b and 452 nm for carotenoids. **(Vernon and Selly, 1966; Hajnal, 2020)**.

2.2. Statistical analysis

Statistical analysis used two-way analysis of variance (ANOVA) **(Snedecor and Cochran, 1980)** where the means separation was carried out using **(Duncan, 1980)**. Multiple range tests and compared using L.S.D test at 0.05 probability level significance was determined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Samples collection

Three types of different symptoms were observed on infected banana trees (cv. Grandnan) grown at Behira, Governorate. Symptoms include typical symptoms of banana bunchy top virus such as yellow at the margins, dark green streaks on leaf veins and midribs **(Fig. 2)**.

Fig. 2. The most obvious symptoms of BBTV on samples collected were dark green streaks on leaves and the plants were stunted.

3.2. PCR detection

BBTV was detected using primers specific to DNA. The expected PCR product (747 bp) amplified from infected symptomatic banana and not from symptomless ones **Fig. 3** out of symptomatic samples, found infected with BBTV. No amplification obtained from negative control or apparently healthy banana trees.

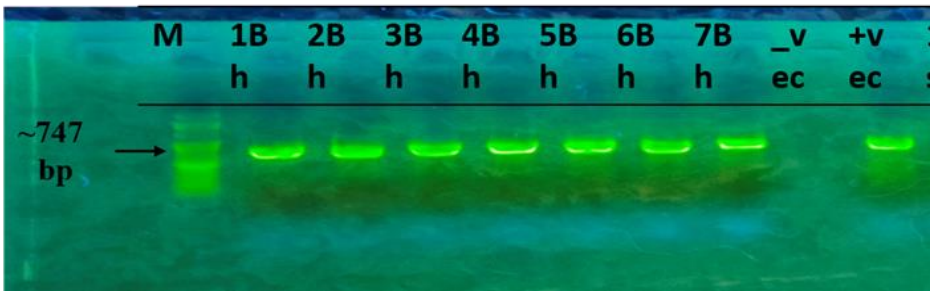

Fig. 3. Agarose gel electrophoresis of PCR product using specific primers of BBTV- coat protein gene to detect banana bunchy top virus (BBTV) in leaves before nano therapy treatments). First lane (M) is a marker. Lane from (1Bh -7Bh) are infected Behira samples. (-ve) Negative control (healthy plant). (+ve) Positive control (infected plant).

3.3. Characterization of chitosan nanoparticles

The transmission electron micrograph illustrates a pseudospherical shape for the synthesized Chi-NPs, and the average size was estimated to be about 36 nm **(Fig. 4)**.

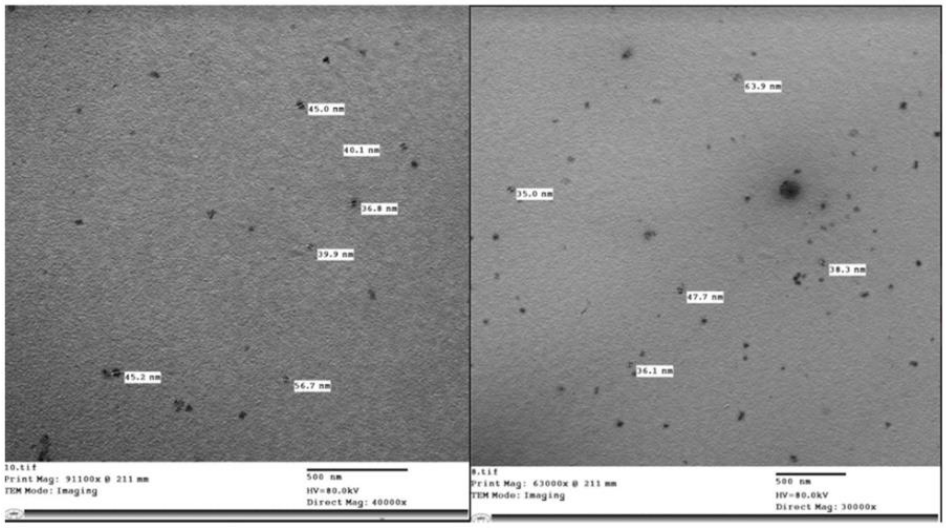

Fig. 4. Transmission electron micrograph illustrating the pseudospherical shape of chitosan nanoparticles (Chi-NPs) with an average size of 36 nm.

3.4. Detection of BBTV after treatments by ELISA

In-vitro experiment designed to evaluate the activities of chitosan nanoparticles on BBTV. The impact of chitosan nanoparticles on BBTV infection was assayed through tissue culture technique **(Fig. 5)**. DAS-ELISA was carried out after 30 days for the presence of BBTV. According to ELISA results **Table 2**, the results obtained showed that the nanomaterials utilized in the treatments effectively suppressed viral replication in the infected explants. This result was confirmed by the study of **El Gamal *et al*. (2022)**. Which mentioned that Chi-NPs were applied therapeutically 48 hours after virus inoculation, they completely inhibited the viral accumulation content and disease infectivity at 300 and 400 mg/L and suggested implication that Chi-NPs ability to suppress plant viruses may be heavily influenced by their particle chemistry and nano physicochemical characteristics nature and bioreactivity, where the antiviral qualities of chitosan appear to be mostly dependent on its nano size. also recorded by **Abdelkhalek *et al*. (2021)** inactivation treatment by Chi-NPs, which resulted in a 100% decrease in the alfalfa mosaic virus. Previous reports have confirmed that Chi-NPs increased the antiviral capabilities against human viruses such as hepatitis C virus38 and HIV39 **(Ramana *et al*., 2014; Loutfy *et al*., 2020)**. It has not yet been determined what mechanisms underlie Chi-NPs' efficacy in preventing plant viruses and other plant diseases. Nanoparticles, which are between 1 and 100 nm in size, offer superior chemical and physical characteristics because of their high surface area to volume ratios in comparison to their bulk materials. **(Osuwa *et al*., 2011 and Bakshi *et al*., 2014)**.

Chi-NPs may have a strong bioreactivity to attract viral RNA with negatively charged phosphate groups in its main chain, which inhibits the spread of the virus **(Xing *et al*., 2009; Mansilla *et al*., 2013)**. Furthermore, it is thought that the positively charged nanoparticles could also target the virus coat protein because all viral

proteins contain negatively charged clusters of glycoproteins. **(Karlin and Brendel, 1988)**. This support our suggestion that chitosan at the nanoscale may be had a role as antiviral activities and that their ability to suppress plant viruses may be greatly impacted by their nano physicochemical properties, particle chemical nature, and bioreactivity.

Table 2. The impact of chitosan nanoparticles on BBTV multiplication based on ELISA results:

Samples	ELISA result (OD)**	+ / - Ve
-Ve control	0.07 e	- ve
+ Ve control	o.40 a	+ ve
Chi-NPs 50	0.20 bc	- ve
Chi-NPs 100	0.16 c	- ve
Chi-NPs 200	0.17 c	-ve

-ve control: un treated healthy plants, +ve control: un treated infected plants, Chi-NPs: Chitosan nanoparticles with concentrations 50, 100 and 200 mg/l. (OD)**, values are the means of three replicates. Means with a common letter are not significantly different ($p > 0.05$).

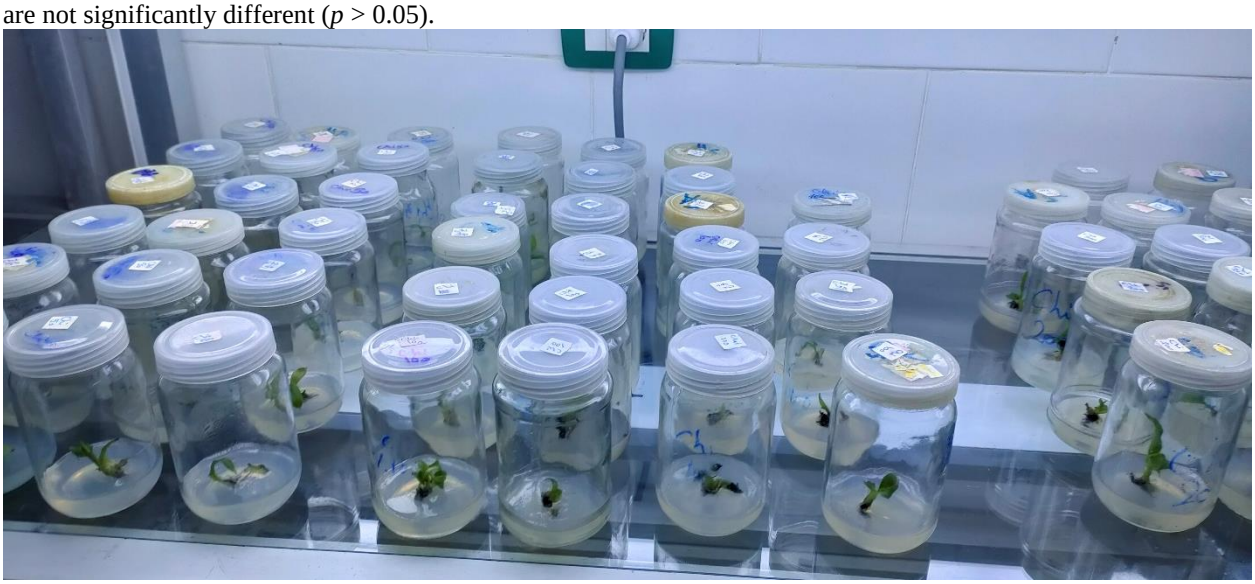

Fig. 5. Overview for treatments by Chi-NPs *in-vitro* with concentrations 50, 100, and 200 mg/l.

3.5. Determination of phytochemicals

3.5.1 Oxidative enzymes

Antioxidant enzymes are known to be essential in halting the detrimental effects of reactive oxygen species (ROS) produced by viral infections, such as damage to plant cells **(Mejía *et al.*, 2013)**. Peroxidase enzymes, for instance, can eliminate H2O2, lessen free radicals, and shield the cytoplasmic membrane **(Isamah *et al.*, 2000)**. The current study demonstrated a significant increase in peroxidase. Peroxidase was reported as an important defense line in plants to control viral infection, such as Pepper yellow mosaic virus in chili pepper **(Gonçalves *et al.*, 2013)**. Recorded increases in antioxidant enzymes, phenolic compounds, and high level of malon-dialdehyde (MDA) may inhibit virus infection. In addition, phenolic compounds led to increase the ability of the plant to inhibit reactive oxygen species may stop virus replication **(Ali *et al.*, 2006)**. It was recorded that chitosan nanoparticles can

act as an immunological method in Plants tea and finger millet plants by triggering the activation of antioxidant defense enzymes **(He *et al.*, 2021; Chandra *et al.*, 2015)**. Through our study we suggested that Chi-NPs promote the plant defense mechanism against virus invasion by increasing the enzymes (peroxidase, polyphenol oxidase) and increasing the total phenolic content. The mode of action underlying the role of chitosan in enhancing plant growth was proposed to be its ability to induce many physiological processes including nutrient uptake, photosynthesis, and cell division as well as plant hormones **(Chakraborty et al., 2020)**.

The results in **Figs 6-8** demonstrated an increase in polyphenol oxidase, peroxidase and total oxidant activity in banana trees infected with BBTV in free medium and which were treated with chitosan nanoparticles compared to healthy banana trees (healthy control).

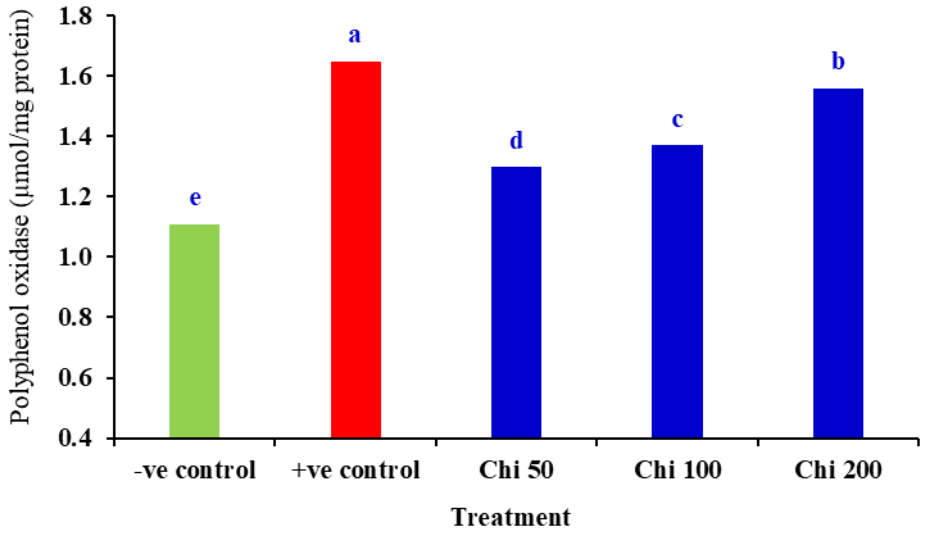

Fig. 6. Effect of chitosan nanoparticles (Chi-NPs) on polyphenol oxidase. -ve control: untreated-healthy plants, +ve control: untreated- infected plants, Chi-NPs: Chitosan nanoparticles with concentrations 50, 100, and 200 mg/l.

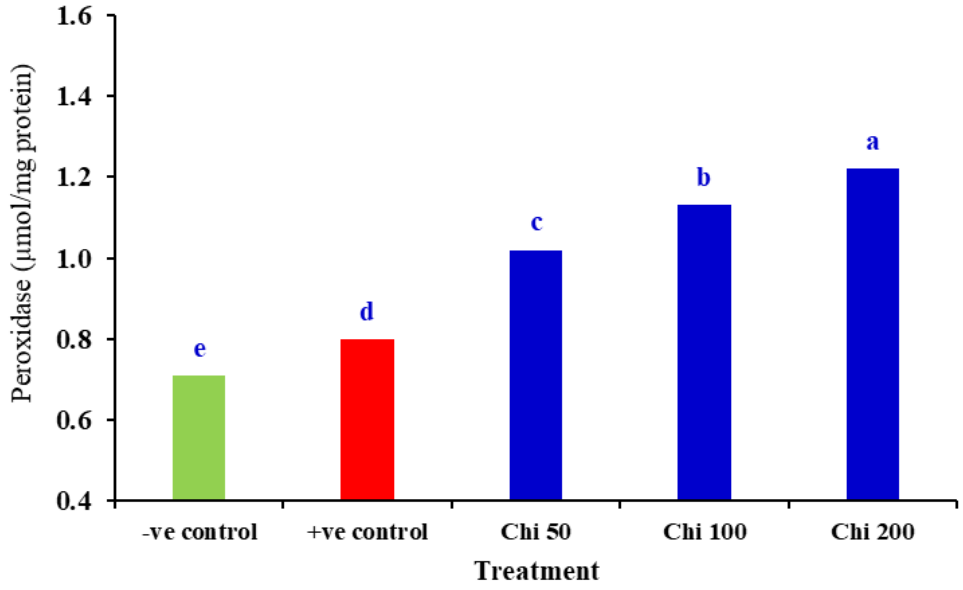

Fig. 7. Effect of chitosan nanoparticles (Chi-NPs) on peroxidase. -ve control: untreated- healthy plants, +ve control: untreated- infected plants, Chi-NPs: Chitosan Nanoparticles with concentrations 50, 100, and 200 mg/l.

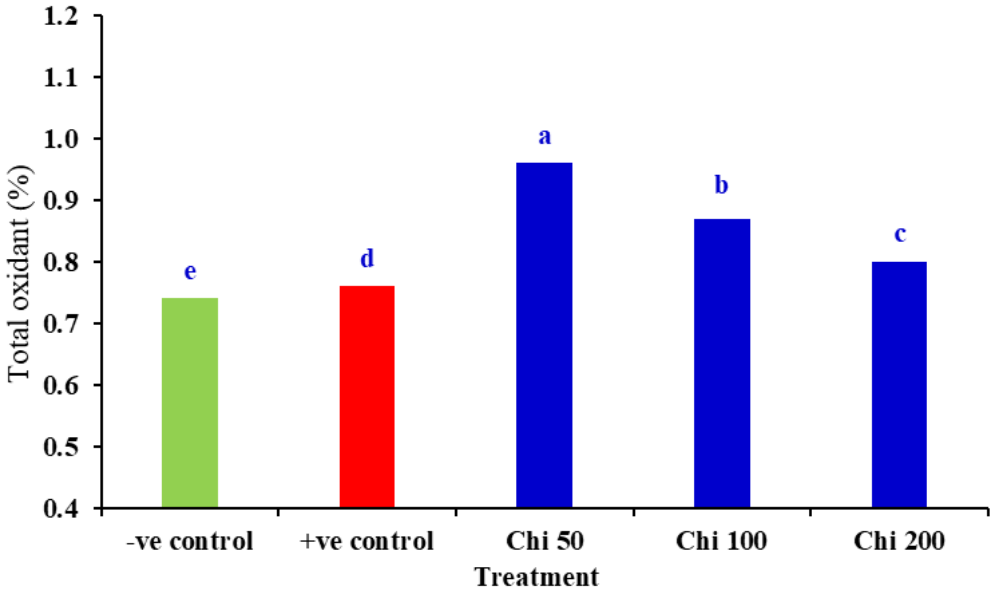

Fig. 8. Effect of chitosan nanoparticles (Chi-NPs) on peroxidase. -ve control: untreated- healthy plants, +ve control: untreated- infected plants, Chi-NPs: chitosan Nanoparticles with concentrations of 50, 100, and 200 mg/l.

3.5.2. **Total phenol, total soluble carbohydrate, protein, and malondialdehyde**

Estimation of total phenols, total soluble sugar, protein and malondialdehyde was carried out in infected samples, and others were treated with chitosan nanoparticles. The data in **Fig. 9** showed that treatments generally led to a significant increase in total soluble carbohydrates. According to **Goodman *et al*. (1965)**. Found that sunflower plants infected with the sunflower chlorotic mottle virus showed comparable results. Additionally, leaves infected with the beet yellows virus had much higher levels of fructose and glucose sugar While some plant viruses may modify the production of carbohydrates or their transport in the leaf tissues, others may not affect either of these processes when infected (**Gaddam *et al.*, 2011)**. According to analysis data estimation of total phenol, there was a significant increase which aligned with **Khalid *et al*. (2011)**. Protein content was significant increase, similar results have been reported by **Radwan *et al*. (2010)**. Protein levels of the BYMV-infected bean leaves were greater than those of the control. There was also a significant increase in MDA In line with a similar finding by **Kaur *et al*. (2024)**. Our data suggest that chitosan nano applications have an effected role in controlling plant viral infection. From our study we propose, to combat virus attacks, plants have developed a number of defense mechanisms, such as plant proteins to inhibit viruses. Defense reactions against plant viruses are mediated by many protein types. And another phytochemicals like carbohydrates MDA and phenols all them are considered a plant defense line against the virus.

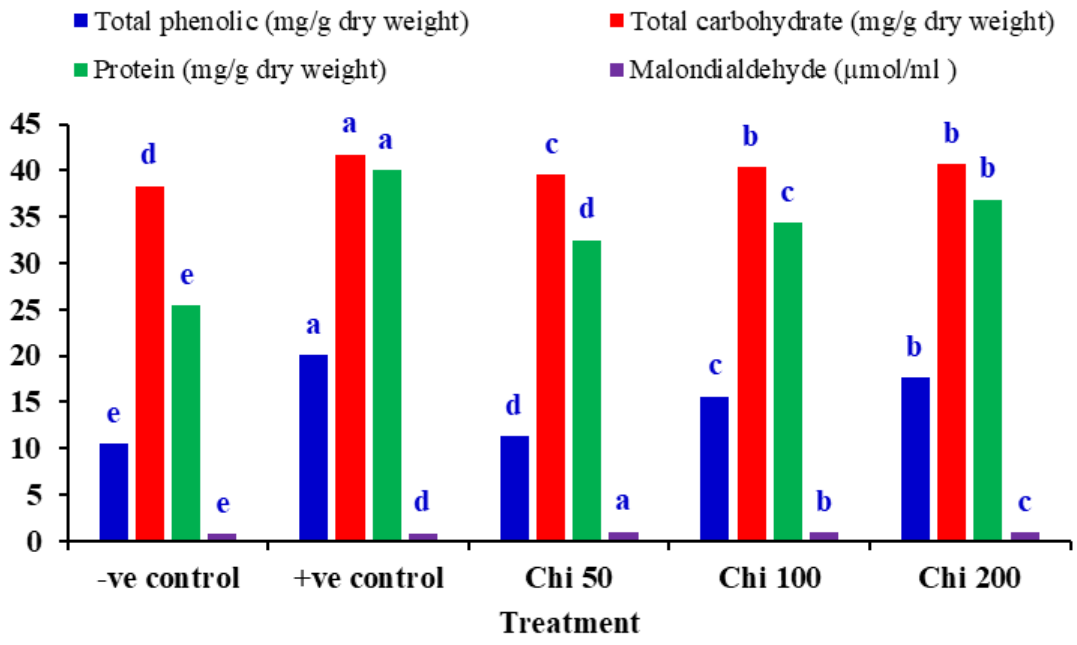

Fig. 9. Effect of BBTV infection and treatments on total phenols, total carbohydrate, protein and malondialdehyde. -ve control: untreated- healthy plants, +ve control: untreated infected plants, Chi-NPs: chitosan nanoparticles with concentrations of 50,100, and 200 mg/l.

3.5.3. Determination of photopigments

Compared to the healthy control photopigments in banana plants treated with three different concentrations of Chi-NPs showed non-significant changes in chlorophyll *a*. Furthermore, the content of carotenoids was significantly increased in the plants treated with 50, 100, and 200 mg/l Chi-NPs compared to the positive control infected plants, on the contrary 100 mg/l Chi-NPs treatment induced a nonsignificant decrease in the content of chlorophyll *b* compared with the healthy controls **(Fig. 10)**. The obtained results of the evaluation of chlorophyll a, b and carotenoids showed that all chlorophyll *a*, *b* and carotenoids decreased in samples infected with BBTV compared with healthy samples and treated samples, but the best concentration was Chi-NPs 50 mg/l, this result is in agreement with **Devi *et al*. (2012)**. It was expected malfunction in chlorophyl synthesis process.

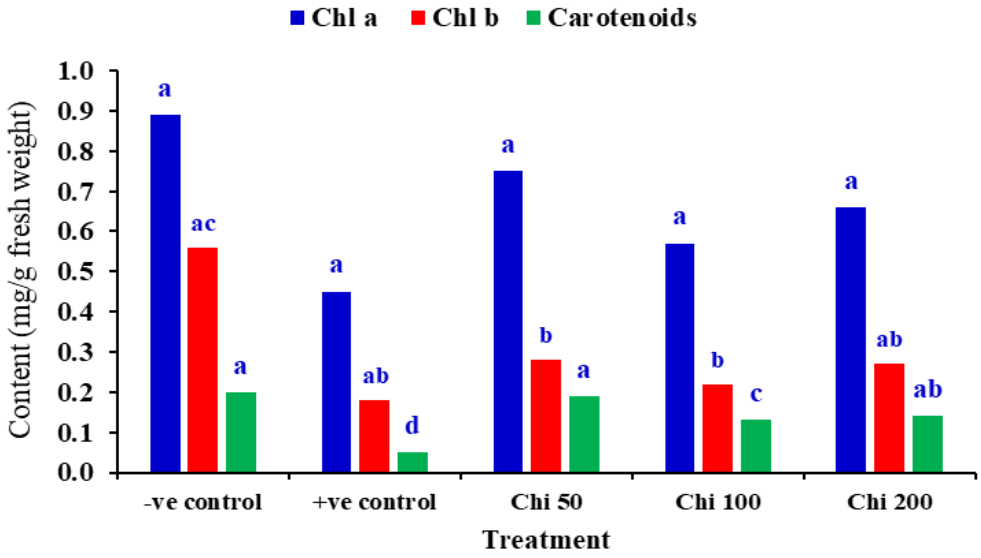

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Fig. 10. Effect of Chi-NPs on Photopigments. -ve control: untreated- healthy plants, +ve control: untreated- infected plants, Chi-NPs: Chitosan canoparticles with concentrations of 50, 100, and 200 mg/l. chl *a*: chlorophyll *a*, chl *b*: chlorophyll *b*.

4. CONCLUSSIONS

In this study, chitosan nanoparticles were used as antiviral to evaluate the efficacy of nano therapy treatments to eradicate the BBTV from the infected banana plantlets. *In-vitro* cultured shoot tip explants of naturally infected mother Grandnan banana plants were treated by different concentrations of Chitosan nanoparticles, which were added to the MS medium for four weeks. With concentrations of 0, 50, 100 and 200 mg/l, after nano therapy treatments, samples of leaves were taken for analysis by ELISA. The results of ELISA analysis proved that the use of chitosan nanoparticles at 50, 100, and 200 mg/l produced the highest virus-free rate of banana Plants. According to the ELISA results, the treatments significantly inhibited virus multiplication in the infected seedlings.

5. REFERENCES

Abdelkhalek, A., Qari, S. H., Abu-Saied, M. A. A. R., Khalil, A. M., Younes, H. A., Nehela, Y. and Behiry, S. I. 2021. Chitosan nanoparticles inactivate alfalfa mosaic virus replication and boost innate immunity in Nicotiana glutinosa plants. Plants, **10**(12):2701.

Abe, N., Murata, T. and Hirota, A. 1998. Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a fungus. Biosci. Biotechnol. Biochem., **62**:661-666.

Alam, M. F., Banu, M. L. A., Swaraz, A. M., Parvez, S., Hossain, M., Khalekuzzaman, M. and Ahsan, N. 2004. Production of virus-free seeds using meristem culture in tomato plant under tropical conditions. J. Plant Biotechnol., **6**(4):221-227.

Ali, S. H., Eisa, S. S. and El-Dougdoug, K. 2006. Role of reactive oxygen species and antioxidants in hyper-sensitive local infected plants. JSSAE, **31**(11):7465-7480.

Bakshi, M., Singh, H. B. and Abhilash, P. C. 2014. The unseen impact of nanoparticles: more or less? Curr. Sci., **106**(3):350-352.

Chakraborty, M., Hasanuzzaman, M., Rahman, M., Khan, M., Bhowmik, P. and Mahmud, N. 2020. Mechanism of plant growth promotion and disease suppression by chitosan biopolymer. Agriculture, **10**:624.

Chandra, S., Chakraborty, N., Dasgupta, A., Sarkar, J., Panda, K. and Acharya, K. 2015. Chitosan nanoparticles: a positive modulator of innate immune responses in plants. Sci. Rep., **5**: 15195.

Chirkov, S. N. 2002. The antiviral activity of chitosan. Appl. Biochem. Microbiol., **38**:1-8.

Clark, M. F. and Adams, N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., **34**(3):475-483.

Davydova, V. N., Nagorskaya, V. P., Gorbach, V. I., Kalitnik, A. A., Reunov, A. V., Solov'Eva, T. F. and Ermak, I. M. 2011. Chitosan antiviral activity: dependence on structure and depolymerization method. Appl. Biochem. Microbiol., **47**:103-108.

Devi, M. C. and Radha, Y. 2012. Induced biochemical changes in the CMV-infected cucurbit plants. Ann. Biol. Res., **3**(2):863-870.

Duncan, J. S. 1980. The superorganic in American cultural geography. Ann. Assoc. Am. Geogr., **70**(2):181-198.

El Gamal, A. Y., Atia, M. M., Sayed, T. E., Abou-Zaid, M. I. and Tohamy, M. R. 2022. Antiviral activity of chitosan nanoparticles for controlling plant-infecting viruses. S. Afr. J. Sci., **118**(1-2):1-9.

Fales, F. 1951. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem., **193**:113-124.

Gaddam, S. A., Kotakadi, V. S. and Saigopal, D. V. R. 2011. Antigenic relationships of Citrus yellow mosaic virus by immunological methods. Asian J. Plant Sci. Res., **1**(1):1-6.

Gonçalves, L. S. A., Rodrigues, R., Diz, M. S. S., Robaina, R. R., Júnior, A., Carvalho, A. and Gomes, V. 2013. Peroxidase is involved in Pepper yellow mosaic virus resistance in Capsicum baccatum var. pendulum. Genet. Mol. Res., **12**:1411-1420.

Goodman, P. J., Watson, M. A. and Hill, A. R. C. 1965. Sugar and fructose accumulation in virus-infected plants: rapid testing by circular-paper

chromatography. Ann. Appl. Biol., **56**(1):65-72.

Hajnal-Jafari, T., Seman, V., Stamenov, D. and Đurić, S. 2020. Effect of chitosan on growth and photosynthetic pigment content in Swiss chard (Beta vulgaris subsp. cicla). Pol. J. Microbiol., **69**(2):235-238.

Hamid, A. and Saleem, S. 2022. Role of nanoparticles in management of plant pathogens and scope in plant transgenics for imparting disease resistance. Plant Protect. Sci., **58**(3):173-184.

Hapsari, L., Rahayuniati, R. F. and Subandiyah, S. 2023. Banana bunchy top virus molecular confirmation and DNA-S phylogenetic of some banana isolates from Indonesia. Hayati J. Biosci., **30**(1):53-64.

He, X., Xing, R., Liu, S., Qin, Y., Li, K., Yu, H. and Li, P. 2021. The improved antiviral activities of amino-modified chitosan derivatives on Newcastle virus. Drug Chem. Toxicol., **44**:335-340.

Hodges, D. M., Delong, J. M., Forney, C. F. and Prange, R. K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissue containing anthocyanin and other interfering compounds. Planta, **207**:604-611.

Hussain, A., Qarshi, I. A., Nazir, H. and Ullah, I. 2012. Plant tissue culture: current status and opportunities. In: Recent Advances in Plant In Vitro Culture. IntechOpen. pp. 1-28.

Isamah, G. K., Asagba, S. O. and Thomas, A. E. 2000. Lipid peroxidation, o-diphenolase, superoxide dismutase and catalase profile along the three physiological regions of Dioscorea rotundata Poir cv Omi. Food Chem., **69**(1):1-4.

Jekayinoluwa, T., Tripathi, L., Tripathi, J. N., Ntui, V. O., Obiero, G., Muge, E. and Dale, J. 2020. RNAi technology for management of banana bunchy top disease. Food Energy Secur., **9**(4):e247.

Karlin, S. and Brendel, V. 1988. Charge configurations in viral proteins. Proc. Natl. Acad. Sci. USA, **85**(24):9396-9400.

Kaur, C., Srivastava, A., Raj, R., Niranjan, A., Lahri, A., Kumar, S. and Raj, S. K. 2024. Physio-biochemical and nutritional alterations in faba bean due to bean yellow mosaic virus infection. Acta Physiol. Plant., **46**(5):54.

Khalid, M., Siddiqui, H. H. and Freed, S. 2011. In-vitro assessment of antioxidant activity of Dalbergia latifolia barks extract against free radicals. AEJSR, **6**(3):172-177.

Kubiriba, J. and Tushemereirwe, W. K. 2003. Occurrence of virus diseases on bananas and plantain in Africa. In: Plant Virology in Sub-Saharan Africa. IITA. pp. 312-320.

Loutfy, S. A., Abdel-Salam, A. I., Moatasim, Y., Gomaa, M. R., Fattah, N. F. A., Emam, M. H. and Kasry, A. 2022. Antiviral activity of chitosan nanoparticles encapsulating silymarin (Sil–CNPs) against SARS-CoV-2 (in silico and in vitro study). RSC Adv., **12**(25):15775-15786.

Loutfy, S., Elberry, M., Farroh, K., Mohamed, H., Mohamed, A. and Mohamed, E. 2020. Antiviral activity of chitosan nanoparticles encapsulating curcumin against Hepatitis C Virus genotype 4a in human hepatoma cell lines. Int. J. Nanomed., **15**:2699-2715.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., **193**:265-275.

Mansilla, A. Y., Albertengo, L., Rodríguez, M. S., Debbaudt, A., Zúñiga, A. and Casalongué, C. A. 2013. Evidence on antimicrobial properties and

mode of action of a chitosan obtained from crustacean exoskeletons on Pseudomonas syringae pv. tomato DC3000. Appl. Microbiol. Biotechnol., **97**:6957-6966.

Mejía, L., Durán, F. D., Chapa, A. M., Torres, I., Cruz, A., González, M. M. and Guevara, R. G. 2013. Oxidative and molecular responses in Capsicum annuum L. after hydrogen peroxide, salicylic acid and chitosan foliar applications. Int. J. Mol. Sci., **14**(5):10178-10196.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., **15**(3):473-497.

Osuwa, J. C. and Anusionwu, P. C. 2011. Some advances and prospects in nanotechnology: a review. Asian J. Inform. Technol., **10**(2):96-100.

Radwan, D. E. M., Fayez, K. A., Mahmoud, S. Y. and Lu, G. 2010. Modifications of antioxidant activity and protein composition of bean leaf due to Bean yellow mosaic virus infection and salicylic acid treatments. Acta Physiol. Plant., **32**:891-904.

Rahayuniati, R. F., Hartono, S., Somowiyarjo, S., Subandiyah, S. and Thomas, J. E. 2021. Characterization of banana bunchy top virus on Sumatra (Indonesia) wild banana. Biodiversitas, **22**(3):1234-1242.

Rahayuniati, R. F., Subandiyah, S., Hartono, S., Somowiyarjo, S., Kurniawan, R. E. K., Prakoso, A. B. and Thomas, J. E. 2021. Recent distribution and diversity analysis on banana bunchy top virus of banana and alternative host in Indonesia. Trop. Plant Pathol., **46**(5):506-517.

Ramana, L. N., Sharma, S., Sethuraman, S., Ranga, U. and Krishnan, U. M. 2014. Evaluation of chitosan nanoformulations as potent anti-HIV therapeutic systems. Biochim. Biophys. Acta, **1840**(1):476-484.

Sauvesty, A., Page, F. and Huot, J. 1992. A simple method for extracting plant phenolic compounds. Can. J. For. Res., **22**(5):654-659.

Schlegel, H. G. 1956. The recovery of organic acid by Chlorella in the light. Planta, **47**:510-526.

Shiragi, M., Baque, M. and Nasiruddin, K. 2008. Eradication of banana bunchy top virus (BBTV) and banana mosaic virus (BMV) from infected plant of banana cv. Amritasagar through meristem culture. J. Plant Stud., **29**:17-41.

Snedecor, G. W. and Cochran, W. G. 1980. Comparisons of two samples. In: Statistical Methods, 7th Edition. Iowa State University Press.

Tchatchambe, N. B. J., Ibanda, N., Adheka, G., Onautshu, O., Swennen, R. L. and Dhed'a, D. 2020. Production of banana bunchy top virus (BBTV)-free plantain plants by in vitro culture. Afr. J. Agric. Res., **16**(3):321-329.

Vargas, M., Macias, I., Guevara, R. G., Rico, E., Ocampo, R. V., Avila, L. and Torres, I. 2020. Nanoparticles as potential antiviral in agriculture. Agriculture, **10**(10): 444.

Vernon, L. P. and Selly, G. R. 1966. The Chlorophylls. Academic Press, New York.

Xing, K., Chen, X. G., Liu, C. S., Cha, D. S. and Park, H. J. 2009. Oleoyl-chitosan nanoparticles inhibit Escherichia coli and Staphylococcus aureus by damaging the cell membrane and putative binding to extracellular or intracellular targets. Int. J. Food Microbiol., **132**:127-133.