MINAR International Journal of Applied Sciences and Technology
ISSN: 2717-8234
Article type: Research Article

Received: 03/12/2023 **Accepted:** 15/01/2024 **Published:** 01/03/2024

USE THE NANO-CHITOSAN TO INHIBIT THE TWO FUNGI ASPERGILLUS NIGER. AND NIGROSPORA SPHAERICA WHICH ISOLATED FROM ZEA MAYS L. LABORATORY

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Abstract

The isolation from Zea mays L. showed two types of fungi Aspergillus niger., Nigrospora sphaerica with a frequency of 38% and 22 %sequentially, Fungi were Isolated diagnosed by keys of taxonomic, Diagnosis of the fungus was assured N. sphaerica depending on the Polymerase Chain Reaction Technology {PCR}. It is the first recording in Iraq/ Karbala Province. Nano-chitosan achieved 100% inhibition rates in vitro**.**

Keywords: *Zea mays L., Aspergillus niger, Nigrospora sphaerica, (PCR), sequencing, Iraq.*

http://dx.doi.org/10.47832/2717-8234.18.1

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Introduction

Environmental pollution with fungi is one of the most important problems facing raw materials used in human food or animal feed which increases this pollution is the availability of suitable conditions for the growth of fungi. Through mismanagement of product operations in the field, starting from the growth and maturity of the crop, harvesting, transportation, processing and storage(Bennett & Klich,2003 and Chulze,2010 and Alramahi, 2021). Fungi produce a many of secondary metabolites during their growth it, like Mycotoxins.The infection occurs either by eating food contaminated with these toxins, by inhaling,by contact(Marshall& PXD,2003).The genus of *Aspergillus* belongs to rank Eurotiales Ascomycota class, Some studies conducted on Poultry feed stored in Nigeria contamination with species of the genus *Aspergillus* This type of fungus produces toxins, the most important of which are aflatoxins(Alasidi,2006).The genus of *Nigrospora* belongs to rank Trichosphaeriales Ascomycota class(Uzor,2015) .The fungus *N. sphaeric*a are considered from the Fungi of a wide family range which cause of damage to several types of trees As well as various plants, it was recorded on trees, grasses, algae and herbaceous plants, It spreads in the air, soil and many different plants as well as various kinds of seeds(Zhang et al,2009). It is widely spread in many parts of the world (Kee et al,2019). It was first scored on the seeds of a plant *Heliocarpus americanus in Brazil (*Bernardi *et al,2021). One of the things that has recently emerged is the use of a component of the cell walls of some organisms It is chitosan that is the main component of protective skin In various crustaceans such as crabs and shrimps (*Kumar et al,2004). The *chitosan* (1,4-glucosamine) it is a chitin derivative, and it has an antifungal effect It is biodegradable and non-toxic it is a bacterial inhibitor and has wide applications in the food industry and, more recently, in the pharmaceutical industry(Sandeep et al,2013).In view of the risk of contamination of corn grain used as poultry feed with aflatoxins the fact that all conditions are favorable for the occurrence of such pollution in Iraq The study aimed to identify the fungus associated with corn grain, *Zea mays* L. In the stores of the Animal Production Department / College of Agriculture in Kerbala/Iraq, and control it by using commercial nano-chitosan in vitro.

MATERIALS AND METHODS

-Isolation & Diagnosis:

Samples of yellow maize grains were collected from the feed store of the Department of Animal Production - faculty of Agriculture - University of Kerbala. With running water the maize grains were washed, With one percent Sodium Hypochlorite the five seeds of maize grains were surface sterilized after that was used sterile distilled water more than once and placed between two filter papers of Whatman (No.10) to get f of excess water.

five seeds were set on the growing medium Potato Dextrose Agar {PDA}. At a degree 25 - 27 °C were incubated for three days to six. By using the Hyphal Tip Technique, the colonies

were cleared. According to Raper Fennell (1965) and Ellis et al (1971) using taxonomic keys for diagnosed fungi morphologically.

Frequencies were calculated for each fungal isolate:

$$\text{The percentage of frequency} = \frac{\text{The number of times fungi appear}}{\text{The number of total colonies}} \times 100$$

Polymerase chain reaction {PCR} Technique was diagnosis assured in center Asco learning/ Baghdad /Iraq.

- Identification of Molecular: It was conducted according to Schloss et.al; (2005) PCR reaction was performed employ Gene Amp, Polymerase chain reaction system 9700 Thermal Cycler {utilized bio system; USA}. By White et.al, (1990) the ITS region was amplified employ ITS1 {forward} & ITS4 {adverse} primer pair supplied by {IDT company, Canada} as described. Taq PCR Pre Mix {Intron, Korea} was utilized in the optimized PCR recipe. About 550bp of the ITS region was amplified using the next program: pre-denaturation at 95°C for 3min.; {denaturation at 94°C for 45sec., annealing at 52°C for 1 min and extension at 72°C for 1 min} for Thirty-five cycles; final extension at 72°C for 7min then holding at 4°C. The amplicons were electrophoresed employ 1.5% agarose gel. According to Zheng et al, (2000) at Gen Bank{NCBI} the nucleotide sequencing results were compared with other Sequences of fungi applying for the Basic Local Alignment Search Tool {BLAST Program}.

-Testing the effect of nanoscale chitosan and Beltanol pesticide in inhibiting the fungi under study.

The effect of Nano-chitosan and Beltanol was tested separately, after mixing them with a sterile PDA medium, at a concentration of (0.1) mg. l^{-1} of Nano-chitosan & 1 $ml.l^{-1}$ before solidification of the medium, Pour the medium into Petri dishes and inoculate with the two fungi under study while chitosan and pesticide were not added to the control treatment, at a temperature of 25- 27 ˚C the samples were incubated at a temperature of 25- 27 ˚C, until the fungus reach the edge of the plate. The results were taken by calculating the average of two orthogonal diameters for each growing colony after the isolated fungi reached the edge of the plate in the control treatment the percentage of inhibition of mycelium growth was calculated according to an equation (Abbott,1925).

$$\text{The percentage of growth inhibition} = \frac{\text{Average of control colony diameter - Average of diameter colony treatment}}{\text{Average of control colony diameter}} \times 100$$

-Statistical analysis: The results were analyzed by Complete Randomized Design {CRD} Duncan's multiple range the means were compared test at a likelihood of 5%, use {SAS} software for statistical analysis (SAS,2012).

Results and Discussion

Isolation & Diagnosis

Isolation results from maize grain showed fungal growth around seeds which cultured on medium (PDA) included *A. niger* with a frequency of 38%, and *N. sphaerica* with a frequency of 22%.

A. niger

According to Someren et al, (1990) fungus growth is rapid 3-4 days, Colonies of the *A. niger* are woolly in the beginning, White then Yellow, after that dark brown to black, characterized of mycelium is growth copious than branched and conidia were divided are spherical, celled was single with a rough wall on the outside, it is three and half to five µm in diameter dark-brown than black in color (Someren et al,1990).

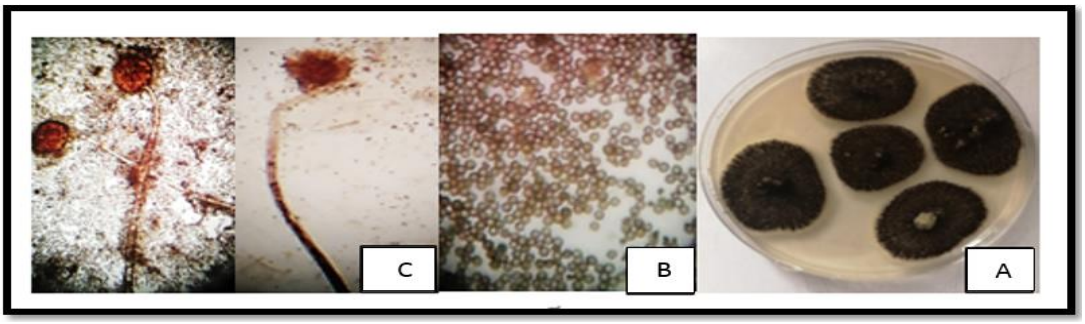

Fig :(1) *A .niger* A- Colony appearance on media B- The conidia of the *A .niger* C- The conidiophore carries conidia with a swollen head it ends.

N. sphaerica

The fungus was characterized by rapid growth and the formation of cottony colonies on the PDA,the color of the colony is white at first then becomes gray with black areas then it turns into a dark grey with the age of the colony the fungal hyphae is divided, conidia are dark black in color, spherical or semi-spherical, slightly shiny, It is carried on the fungal hyphae, specifically at the end of the conidiophore At the top there is a slit for the exit of germs, the dimensions of which are 7.9-10.7 x 10-12.1 micrometers. **(Figure 2)** These characteristics matched the specifications of the fungus N. sphaerica, which he mentioned Song et al, (2016)and Wong et al,(2017)and Hao et al, (2020).

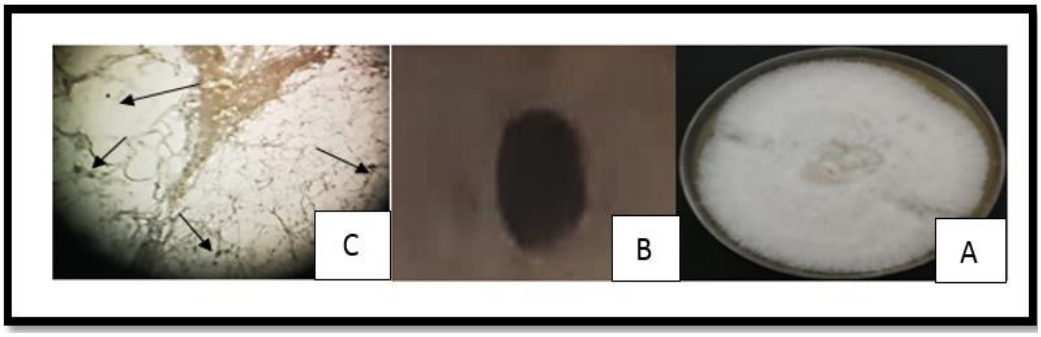

Fig :(2) *N. sphaerica*- Colony appearance on media B- The conidia of the *N. sphaerica* C- Mycelium with a number of conidia.

-Identification of *N. sphaerica* by PCR Technique:

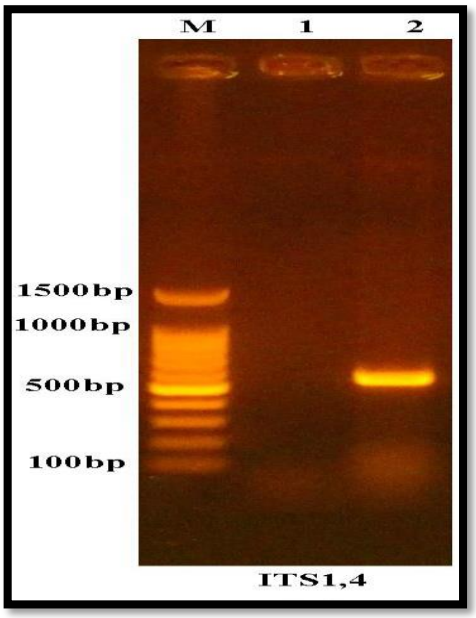

Fig :(3) The bundles formed from the electrophoresis and in sizes (Base pairs, pb) Pinned to the left side of the DNA ladder.

Nitrogen base sequence analysis showed that isolate {No. 2} was belongs to the fungus *N. sphaerica.* The sequence was placed in the database (Genbank) National Centre of Biotechnology Information (NBCI) Registered at the serial number of OR421197.1.

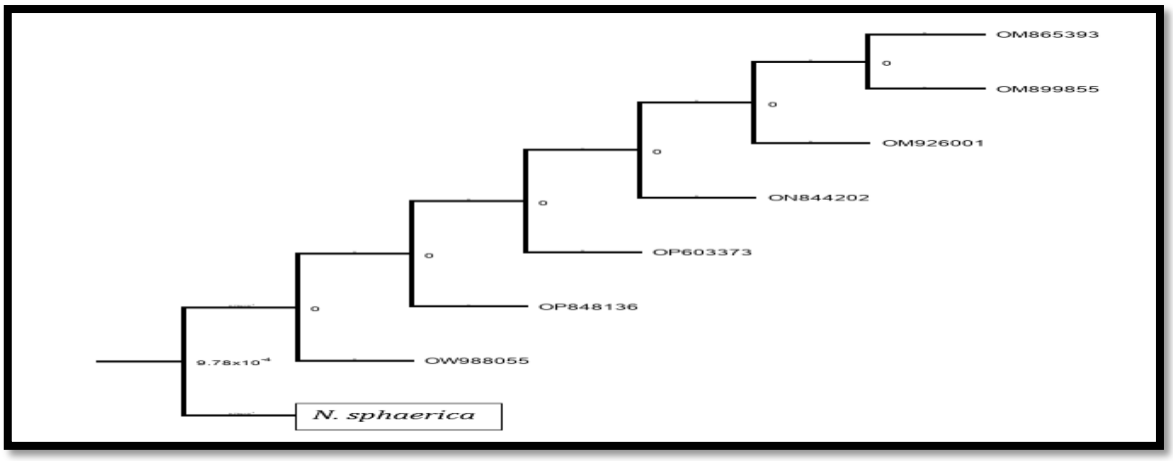

Fig: (4) Genetic tree of *N. sphaerica*

Similarity rate was 100% for the fungus *N. sphaerica* using BLAST analysis, It is considered is the first register of it on maize grains under study in karbala / Iraq.

The polymerase chain reaction (PCR) technique has been employ in prior experiments due to its high fineness in diagnosing organisms of various, including fungi, Microorganism cultures, even if they belong to the same group, differ genetically in their growth characteristics and morphological characteristics, Variation may be due to the response of farms or isolates to environmental conditions (Muhialdiyn and Gigan, 2013).

-The effect of Nano-chitosan in inhibiting fungi under study

Nano-chitosan achieved high inhibition rates against the two fungi under study at laboratory conditions reached 100% compared to the control treatment(Figure, 5).

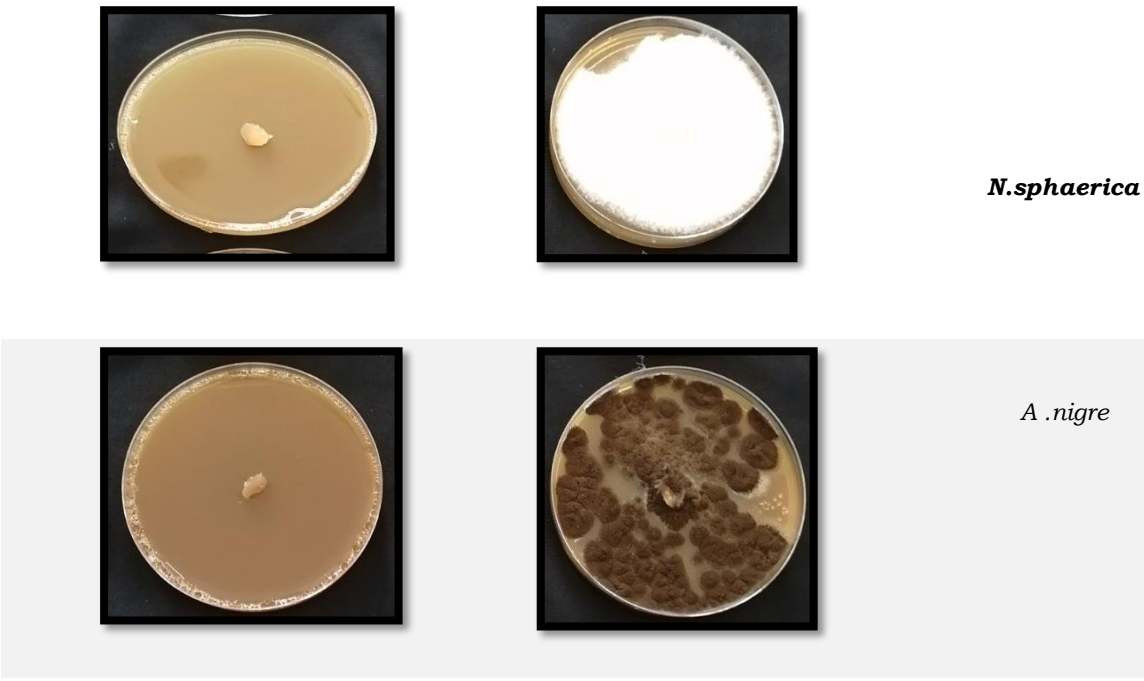

Fig: (5) Effect of chitosan nanoparticles in the diagonal growth of the tested fungi.

Table (1) shows the effectiveness of the Nano-chitosan in inhibiting mycelial growth of fungi isolated from seed corn at 100% concentration Which reached 100% Compare with Control for all fungi tested.

Table (1) Effect of the Nano-chitosan on colony diameter growth of the tested fungi (0.1 mg. L^{-1}).

Concentrations mg.L^{-1} / Fungi	**0**	0.1	**Average fungicidal effect**
0	**0 c**	**100 a**	**50 b**
N.sphaerica	**0 c**	**100 a**	**50 b**
A .nigre	**0 c**	**100 a**	**50 b**
Effect of average concentrations	**0 c**	**100 a**	

*Numbers bearing different letters indicate that there are significant differences between them at the probability level of 5% according to Duncan's multinomial test.

Table (2) shows the effectiveness of the chemical pesticide Beltanol in inhibiting mycelial growth of fungi isolated from seed corn at 100% concentration Which reached 100% Compare with Control for all fungi tested.

Table (2) Effect of the chemical pesticide Beltanol on colony diameter growth of the tested fungi (ml. L-1).

Concentrations m.L^{-1} / Fungi	**0**	1	**Average fungicidal effect**
0	**0 c**	**100 a**	**50 b**
N.sphaerica	**0 c**	**100 a**	**50 b**
A .nigre	**0 c**	**100 a**	**50 b**
Effect of average concentrations	**0 c**	**100 a**	

*Numbers bearing different letters indicate that there are significant differences between them at the probability level of 0.05 according to Duncan's multinomial test.

Nano chitosan showed high efficiency in inhibiting the fungal growth of *N. sphaerica* and *A.niger fungi.* on *Zea mays* L. which it stored in the laboratory, compared to the comparison treatment, which is consistent with Jeon et al. (2002) of the results and Nadarajah (2005). Previous studies indicated that chitosan forms thin films that are used as coatings around grains, and it increased the ability to store perishable foods when they were coated with chitosan, this reduces the economic losses that occur when storing food (El Ghaouth et al., 1991 & Zhang & Quantick, 1998). This is somewhat similar to the interpretation of Shukla et al. (2013). Nano chitosan is of greater biological value compared to regular chitosan, as it possesses superior physical and chemical properties such as high surface area. In an experiment conducted by Pilon et al. (2015) nanoscale chitosan was used A material for coating fresh apples, Nanoparticles with a diameter of 110 nanometers showed high effectiveness in inhibiting the growth of microorganisms .

It is concluded from the results of the current study that the first report of *Nigrospora sphaeric*a fungus which isolated from (*Zea mays* L) seeds in Karbala of Iraq.

References

Abbott W S (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:

Alasadi, S. H. A.(2006). Effect of filtrates of some fungi isolated from poultry feed on the growth of chicken chicks in some areas of Karbala. Thesis, Faculty of Education, University of Kerbala/Iraq.

AlRamahi, A. H. H.(2021). Protection of maize crop by using some nanomaterials against infection with the fungus Aspergillus flavus and its effect on reducing aflatoxin production. Thesis, College of Agriculture, University of Baghdad/Iraq.

Bennett, J. W, and Klich, M. (2003). Mycotoxins. ClinMicrobiol Rev 16 (3): 497 - 517.

Bernardi, C.; Busso, C.; **Borin,R.C.; Mazaro, S. M. ; aburo, R. S. S. (2021).** The First report of *Nigrospora sphaerica* Associated with *Heliocarpus americanus* Seeds in Brazil. Floresta Ambient, 28(1):1-3.

Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage:a review. *Food Additives and Contaminants*, 27(5), 651-657.

El Ghaouth, A.; Arul, J.; Ponnampalam, R., and Boulet, M. (1991). Chitosan coating effect on storability and quality of fresh strawberries. *Journal of food science*, *56*(6), 1618-1620.

Ellis, M. B. (1971). Dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew suvey. England.

Hao, Y.; Janith, H.V.S.; Aluthmuhandiram, K.W.T.C.; Manawasinghe, I.S.; Mei Liu, X.; Hyde, M.K.D.; Phillips, A.J.L.& Zhang, W. (2020). *Nigrospora* Species Associated with Various Hosts from Shandong Peninsula, China. MYCOBIOLOGY, 48 (3): 169–183.

Jeon, Y. J.; Kamil, J. Y.; and Shahidi, F. (2002). Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and food chemistry*, *50*(18), 5167-5178.

Kee, Y. J.; Hafifi, A. B. M.; Huda-shakirah, A.R.; Wong, K. L.; Jin, X.L.; Nordahliawate, M. S. S.; Zakaria, L.; Mohd, M.H. (2019). First reportof reddish brown spot disease of red-fleshed dragon fruit (Hylocereus polyrhizus) caused by Nigrospora lacticolonia and *Nigrospora sphaerica* in Malaysia.Cropprotect. 122,165-170.

Kumar, M. R.; Muzzarelli, R.; Muzzarelli, C.; Sashiwa, H. and Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical reviews*, *104*(12), 6017-6084.

Mohiuddin, M. O. and Jigan, R. A. (2013). Enzyme production of Polgalactyronase from heat-prone and enzyme-producing mold Aspergillus niger and identification of the most productive isolate. Iraqi Agricultural Sciences Journal - 44 (1), pp. 97-105.

Nadarajah, K. (2005). Development and characterization of antimicrobial edi-ble films from crawfish chitosan.

Pilon, L.; Spricigo, P. C.; Miranda, M.; de Moura, M. R.; Assis, O. B. G.; Mattoso, L. H. C. and Ferreira, M. D. (2015). Chitosan nanoparticle coatings reduce microbiAlgrowth on fresh-cut apples while not affect-ing quality attributes. *International Journal of Food Science & Tech-nology*, *50*(2), 440-448.

Raper, K.B. and Fennell, D.I. (1965). The Genus Aspergillus. Williams and Wilkins, Philadelphia, 686 p/Taxonomy and to industrial Application . Plenum press, London ; 261-279.

S.A.S. (2012). Statistical analysis system, users guide. Statistical. Version 9,1 nd SAS. Instituted Inc.Cary, N.C. USA.

Sandeep, A.; Sangameshwar, K.; Mukesh, G.; Chandrakant, R. and Avinash, D. (2013). A brief overview on chitosan applications. *Indo American Journal of PharmaceuticAlResearch*, *3*(12), 1564-4574.

Schloss, PD.; Hay GA, Wilson DB et. al. (2005). Quantifying bacterial population dynamics in compost using 16S rRNA gene probes. Appl. Microbiol. Biotechnol., 66: 457-463.

Shukla, S. K.; Mishra, A. K.; Arotiba, O. A. and Mamba, B. B. (2013). Chi-tosan-based nanomaterials: A state-of-the-art review. *International Journal of Biological macromolecules*, *59*, 46-58.

Someren, K.; Kester, H.C.M.; Samson, R.A., and Visser, J. (1990). Variations in Pectolytic Enzymes of the Black Aspergilli: A Biochemical and Genetic Approach. In: Modern Concepts in Penicillium and Aspergillus Classification, Samson, R.A. and J.I. Pitt (Eds.). Plenum Press, New York.

Song, J. J.; Pongnak, W.and Soytong, K.(2016). AntifungalActivity of Endophytic Fungi fromPalm TreesAgainst CoffeeAnthracnose Caused by *Colletotrichum Coffeanum*. International Journal of Agricultural Technology, 12(3):527-539.

Uzor, P.F.; Ebrahim, W.; Osadebe.; P.O. (2015). Metabolites from Combretum dolichopetalum and its associated endophytic fungus *Nigrospora oryzae* – evidence for a metabolic partnership. Fitoterapia 105: 147–150.

Wang, Z.H.; Zhao, Z.X.; Hong, N.; Ni, D.; Cai, L.; Xu, W.X.; Xiao, Y. N.; (2017). Characterization of causal agenst of a novel disase inducing Brown black spots on tender tea leaves in china. plant. Dis .101,1802-1811.

White, TJ.; Bruns T; Lee S, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and

Applications, eds. Innis, M. A.; Gelfand, D. H.; Sninsky, J. J. and White, T. J. Academic Press Inc., New York. pp. 315-322.

Zhang, D., and Quantick, P. C. (1998). Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. *The Journal of Hor-ticultural Science and Biotechnology*, *73*(6), 763-767.

Zhang, Qi-Hui; Tian, Li; Zhou, Lian-Di; Zhang, Ying; Li, Zhi-Feng; Hua, Hui-Ming; Pei, Yue-Hu (2009). "Two new compounds from the marine Nigrospora sphaerica". *Journal of Asian Natural Products Research*. **11** (11): 962–6.

Zheng, ZS, Schwartz, S, Wagner, L and Miller, W. (2000). A greedy algorithm for aligning DNA sequences *Journal of Computational Biology*, 7: 203-214.

SAS. Statically analysis system (2012). Users guide. Statistical. 1th ed. SAS. L. nst. Inc. Cary. N.C. USA.