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Termiticidal activity of mycosynthesized silver nanoparticles from *Aspergillus fumigatus* BTCB15

Actividad termiticida de nanopartículas de plata micosintetizadas de *Aspergillus fumigatus* BTCB15

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Abstract

Termites, due to their feeding behavior are exceedingly disastrous to the community affecting not only the infrastructure of buildings but also responsible in converting fields into barren lands. Though insecticides are used to control termites but it is not an ecofriendly approach. In the current study silver nano particles were produced from *Aspergillus fumigatus* BTCB15 and its effect was observed in controlling termites. Feeder blocks were treated with different concentrations (0.1, 1, 5, 10 and 15ppm) of nanoparticles and fed to the subterranean termites of *Coptotermes* sp. for two weeks. Among all the concentrations used 15ppm was the most effective in which 100% mortality was achieved on the 9th day and the mean weight loss of block was 3.3% as compared to control which gave 32.2% mortality rate and mean weight loss was 29%. Among all the concentrations used 0.1 ppm treated blocks were also affected by fungus which showed that higher concentrations were also effective in inhibiting fungal growth. Conclusively, this study showed that mycosynthesized silver nanoparticles are very effective in protecting the wood from damaging not only from termite but also from wood deteriorating fungus. Further study should be undertaken to bring this approach at commercial scale.

Keywords: *Aspergillus fumigatus*, silver nanoparticles, termites, wood protection.

Resumen

Las termitas, debido a su comportamiento de alimentación, son extremadamente desastrosas para la comunidad y afectan no solo la infraestructura de los edificios, sino que también son responsables de convertir los campos en tierras áridas. Aunque los insecticidas se usan para controlar las termitas, no es un enfoque ecológico. En el estudio actual, se produjeron nanopartículas de plata de *Aspergillus fumigatus* BTCB15 y se observó su efecto en el control de las termitas. Los bloques de alimentación se trataron con diferentes concentraciones (0,1, 1, 5, 10 y 15 ppm) de nanopartículas y se alimentaron a las termitas subterráneas de *Coptotermes* sp. por dos semanas. Entre todas las concentraciones utilizadas, 15 ppm fue la más efectiva en la cual se logró el 100% de mortalidad en el noveno día y la pérdida de peso promedio del bloqueo fue de 3.3% en comparación con el control que dio una tasa de mortalidad de 32.2% y la pérdida de peso promedio fue de 29%. Entre todas las concentraciones utilizadas, los bloques tratados con 0.1 ppm también se vieron afectados por hongos que mostraron que concentraciones más altas también fueron efectivas para inhibir el crecimiento de hongos. En conclusión, este estudio demostró que las nanopartículas de plata micosintetizadas son muy efectivas protegiendo la madera de daños no solo de termitas sino también de hongos que deterioran la madera. Se deben realizar más estudios para llevar este enfoque a escala comercial.

Palabras clave: *Aspergillus fumigatus*, nanopartículas de plata, termitas, protección de la madera.

1 Introduction

Termites like any other organism have essential role in ecological cycle as it breaks down the detritus and enrich the soil with essential nutrients. However, quiet often their feeding behavior can be very detrimental causing huge damage to fields, homes and buildings (Orkin, 2016). The economic loss caused by termites is increasing annually, which was almost 11 billion in United States during 1999 (Su, 2002). This also includes repair cost of buildings and cost of termiticides like imidacloprid, fipronil, fevalerate, cypermethrin and chlorpyrifos.

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According to a previous study the annual cost of termites control has reached $22 billion around the globe which is increasing by 50% each year (Su, 2002). In USA alone termites control costs €175 million annually (Govorushko, 2019).

To avoid the side effects caused by the usage of insecticides such as production of detrimental Persistent Organic Pollutants (POPs), another approach is the use of metal nanoparticles that at very low concentrations can eradicate termites (Singh *et al*., 2012). The preparation of silver nanoparticles is done by chemical approach and physical approach but these methods have adverse aspects that includes toxicity, high cost and non-eco-friendly nature. For the production of eco-friendly, nontoxic and cheap nanoparticles recently scientists have developed nanoparticles by bacteria (He *et al*., 2007), fungus (Husseiny *et al*., 2007) and plants (Zheng *et al*., 2017). In the present study wood is treated with mycosynthesized nanoparticles which is an eco-friendly and cost-effective method.

Globally, fungi have caught attention of the researchers as effective nanofactories globally. Mycogenic route for nanoparticles synthesis is well recognized due to its several remarkable features which are well documented. Extracellular enzymes produced by fungi play important role in the synthesis of nanoparticles. Another economical aspect of mycosynthesis is that fungi have simple nutritional requirements whereas, biomass produced by fungi is large and easy to handle as compared to bacteria (Gade *et al*., 2008; Rai *et al*., 2009). It can be sustained under high agitation and flow pressure (Saha *et al*., 2010). Various studies reporting nanoparticle synthesis from different fungi are *Penicillium* (Shaligram *et al*., 2009; Hemath Naveen *et al*., 2010), *Aspergillus* sp. (Bhainsa *et al*., 2006; Vigneshwaran *et al*., 2007; Verma *et al*., 2010), *Cladosporium* sp. (Durán *et al*., 2005; Basavaraja *et al*., 2008; Balaji *et al*., 2009) and *Trichoderma* sp. (Vahabi *et al*., 2011; Singh and Raja, 2011).

Among so many metals nanoparticles, the most extensively used are silver nanoparticles nanoparticles (AgNPs) because of their antimicrobial activity. AgNPs have wide applications and are used in different cosmetic products, water treatment processes, textile industries, drug formulations etc. Silver nanoparticles are added to paints to insure aseptic environment and in surface coatings in neurosurgical shunts, cathetors, bone cementing and other implants. These are also used in wound dressing and to prevent infection and enhance wound healing (Chaloupka *et al*., 2010; Serrano-Niño *et al*., 2019). This shows the efficiency, reliability, and eco-friendly nature of nanoparticles. Termicidal property of metal nanoparticles was reported by (Terzi *et al*., 2016) which showed that 1% (10,000ppm) nano-CuO and 1% (10,000 ppm) nano-B_2O_3 were effective against subterranean termites. Similarly other studies undertaken by (Mantanis *et al*., 2014) which showed that concenteration of 20,000 ppm Nano zinc oxide is an effective termicide. Whereas (Kartal *et al*., 2009) showed that at 10,000 ppm nano zinc and nano boron and (Green *et al*., 2007) showed that at 5000ppm nano-zinc were highly effective against termites. In these studies very high concentrations of nanoparticles were subjected for termite inhibition which could lead to the accumulation of metal in the environment effecting the biota. The objective of this study was to observe the termiticidal activity of mycosynthesized silver nanoparticles produced from *Aspergillus fumigatus* in very low concentrations.

2 Materials and methods

2.1 Microorganism

For obtaining myco-synthesized silver nanoparticles fungal isolate *Aspergillus fumigatus* BTCB15 was obtained from Biotechnology Culture Collection Bank. The isolate was cultured onto potato dextrose agar (PDA) at temperature 25 °C.

2.2 Silver Nanoparticle Synthesis

2.2.1 Biomass preparation

Synthetic liquid media was prepared according to Hemath Naveen *et al*. (2010), comprised of (gL^{-1}) KH_2PO_4, 7.0: K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The media was inoculated with the fungi spores (10^8 spores/mL) measured with cell counting chamber and incubated at 25 °C for 3 days in a rotary shaker (120 rpm). After the incubation time period biomass obtained was filtered through Whatman filter paper (0.45 μm).

2.2.2 Silver Nanoparticles Synthesis

To the obtained 7g of biomass 100 ml of distilled water was added and incubated at 25 °C for 3 days at 120 rpm. The cell free extract (CFE) was obtained by filtering the mixture through Whatman filter paper no. 1. To the CFE, 1mM $AgNO_3$ was added in 1:1 ratio (v/v). The mixture was incubated at 25 °C till the change in color of the solution became stable that indicated completion of reaction (Basavaraja *et al*., 2008).

2.3 *Characterization of mycosynthesized silver nanoparticles*

In order to confirm the reduction of silver ions to silver nanoparticles U.V visible spectroscopic analysis was done and excitation peaks were observed in the range of 300-800 nm. The particle size was analyzed by the particle size analyzer BT-90 (Kim *et al*., 2017). The presence of functional groups as capping agents with mycosynthesized silver nanoparticles was done by FTIR analysis in the range between 4500-500 cm^{-1} (Velu *et al*., 2017).

2.4 *Wooden blocks treatment*

Feeder blocks of 3/4 by 3/4 by 3/4 cm were dipped for 24 hours in five groups of different concentrations i.e. 15ppm, 10ppm, 5ppm, 1ppm and 0.1ppm of mycosynthesized silver nanoparticles and one group as control where blocks were dipped in distilled water (Terzi *et al*., 2016).

2.5 *Termite studies*

Wooden blocks were dried at 50 °C for three days and weighed before starting the experiment. The blocks were placed in glass beakers of 250ml and 75 worker and 15 soldier termites were added and observed for two weeks. The blocks were kept moist during the experiment and were held at a constant temperature (28 °C). After 14 days the blocks were dried again under same conditions. The blocks were weighed and the mean weight loss of wood was calculated and the rate of termite mortality was recorded (Mantanis *et al*., 2014).

2.6 *Statistical analysis*

Statistical Package for Social Science (SPSS) software was used to perform statistical analysis. Each value was the mean of three replicates ± standard error. Significant differences ($p \leq 0.05$) between the treatments were analyzed by Duncan multiple range test.

3 Results and discussion

3.1 *Synthesis*

During this study silver nanoparticles were mycosynthesized by using *Aspergillus fumigatus* similar to the study proposed by Bhainsa *et al*. (2006).

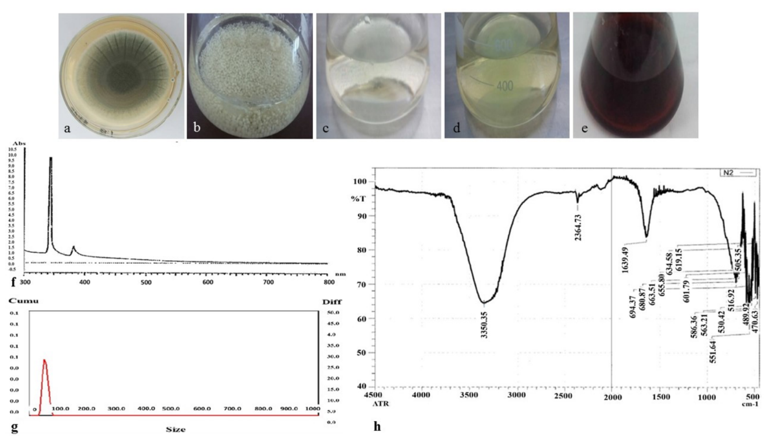


Fig. 1. Silver nanoparticles synthesis and characterization. a) *Aspergillus fumigatus* culture plate b) Biomass production from *Aspergillus fumigatus* c) Cell free extract filtered from biomass d) After addition of silver nitrate color of CFE changed colour indicating the synthesis of AgNPs e) The color further changed to dark brown after 24 hours and became stable f) UV visible spectrum of AgNPs biosynthesized from *Aspergillus* sp. g) Graph indicating the particle size of biosynthesized AgNPs using BT-90 particle size analyzer h) FTIR analysis of AgNPs biosynthesized from *Aspergillus* sp.

In this study A. *fumigatus* was selected due to its specific properties like, extracellular reduction of silver ions and rapid formation of silver nanoparticles as compared to other species as described by Ranjbar Navazi *et al*. (2010). Upon incubation of the CFE and AgNO3 solution under optimum conditions of *Aspergillus fumigatus*, a frequent change in colour from colourless to yellow which later converted into dark brown within 24 hours was observed (Fig. 1a-e). This change in colour indicated that silver was reduced into silver nanoparticles due to the presence of fungal enzymes (Vanaja *et al*., 2015).

3.2 Characterization

The U.V Vis spectrum of synthesized AgNPs presented peaks at 386 nm that confirmed the formation of silver nanoparticles (Fig. 1f). AgNPs showed peak in the range of 360-460 nm which is contrary to the studies in literature (Bhainsa *et al*., 2006; Kathiresan *et al*., 2009) which showed peaks at 420nm and 430nm. The particle size measured was 54.61nm (Fig. 1g). Fourier transform infrared spectroscopy revealed the presence of amines with medium intensity and stretching vibrations at 3350.35 cm^{-1}, nitriles having medium intensity and stretching vibrations at 2364.73 cm^{-1} and alkene (C=C) at 1639.49 cm^{-1} having stretching vibrations with variable intensity. Bands observed at 694.87 cm^{-1}, 680.87 cm^{-1}, 663.51 cm^{-1}, 655.80 cm^{-1}, 634.58 cm^{-1}, 619.15 cm^{-1}, 601.79 cm^{-1} showed the presence of alkyl halide (C-Cl) with strong intensity and stretching vibrations and 586.36 cm^{-1}, 563.21 cm^{-1}, 551.64 cm^{-1}, 530.24 cm^{-1}, 516.92 cm^{-1}, 505.35 cm^{-1}, respectively. Alkyl halide (C-Br) having strong intensity and stretching vibrations were also present (Fig. 1h). FTIR analysis indicated the presence of amines, nitriles, alkenes and alkyl halides similar to the study presented in (Hemath Naveen *et al*., 2010). These capping agents help in detection of nanoparticles as these are present to control the growth of nanoparticles (Raheman *et al*., 2011).

3.3 Termiticidal activity of AgNPs

In the present study, different concentrations of myco-synthesized silver nanoparticles were applied to the wooden blocks and treated wooden blocks were fed to termites for two weeks in order to observe the termiticidal activity of the mycosynthesized silver nanoparticles. Figure 2(a) illustrates that for group I (control) mortality rate was only 32.2% during the study period. The mortality rate for group II (0.1ppm) reached 100% on 13thday of the study period (Fig. 2b). Mortality rate for both group III (1ppm) and IV (5ppm) was 100% on 10thday (Fig. 2c, d).

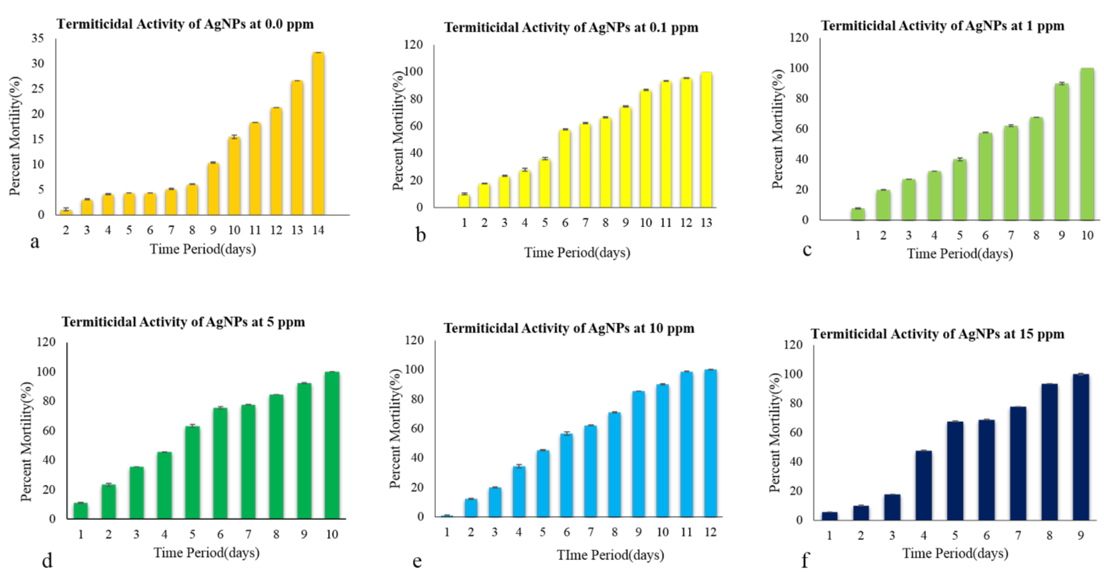


Fig. 2. Termiticidal activity observed on samples treated by a) control b) 0.1ppm c) 1ppm d) 5ppm e) 10ppm f) 15ppm during 14 days of study.

The termiticidal activity of 10 ppm AgNPs (group V) was lower than 5ppm and 1ppm, and 100% mortality was achieved on 12thday of the study period (Fig. 2e). Termiticidal activity enhanced with increase in concentration of AgNPs. The mortality rate for group VI (15ppm) reached 100% within 9 days (Fig. 2f). This is the first study to show effective termiticidal activity of silver nanoparticles at very low concentration. Different studies conducted for termite inhibition showed that nano-CuO nanoparticles of 1% (10,000ppm) caused 15.15% termite mortality during the two week study whereas 1% (10,000 ppm) nano-B_2O_3 was effective against subterranean termites by reaching 100% mortality rate within a week (Terzi *et al*., 2016). Whereas in other study in (Mantanis *et al*., 2014), 2% (20,000 ppm) nano zinc borate and nano zinc borate plus acrylic emulsion were most effective and caused 100% mortality during 4 weeks study. Another study proposed that 1% (10,000ppm) nano zinc and nano boron were effective termiticide with 100% and 31% mortality (Kartal *et al*., 2009) whereas 70-76% termite mortality with 0.5% (5000ppm) nano-zinc was highly effective against termites (Green *et al*., 2007).

3.4 Physical evaluation of blocks and termites at various AgNPs concentrations

3.4.1 Physical evaluation of termites

Physical evaluation of termites (Isoptera) was conducted on the bases of hedonic scale having values from 9 to 1 on the bases of their condition. These values were based on extreme like to extreme dislike. Where the value 9 represents sound and 8 to 7 are slightly damaged, 6 to 4 are moderately damaged and 3 to 1 are heavily damage. For this evaluation opinion of a panel comprising of 20 persons of LCWU was taken. The evaluation indicated that the termites fed on blocks with higher concentration of AgNPs, showed sever signs of damage as compared to those fed with less concentrations. The effect worsened with the passage of time and the gut area was affected badly that could be due to the silver nanoparticles. Whereas the condition of the termites of control remained acceptable. The condition of the termites fed with 15 ppm AgNPs coated blocks was rated as highly damaged from the very first day and further worsened with the passage of time.

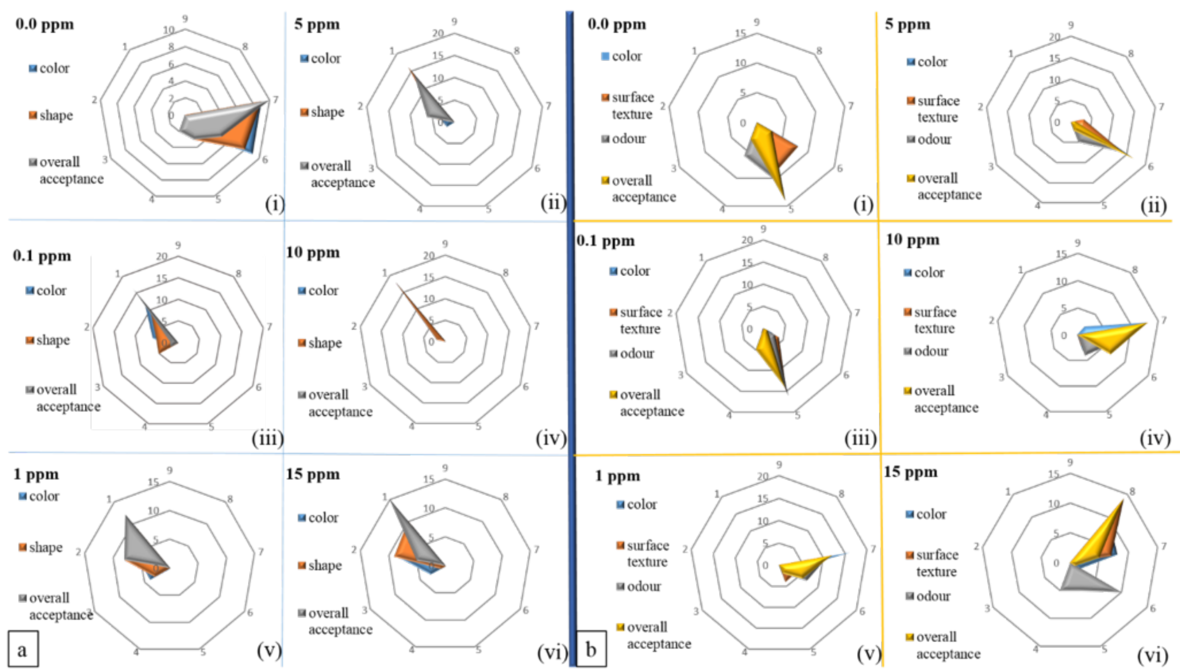


Fig. 3. Physical evaluation of Termites and blocks. Termites on day of termination of experiment a (i) control on 13thday of study a (ii) 0.1ppm terminated on 13thday a (iii) 1ppm terminated on 12thday of study a (iv) 5ppm terminated on 10thday of study a(v) 10ppm terminated on 12thday a (vi) 15 ppm terminated on 9thday of study. Right side b (i-vi) blocks treated by different concentrations on 14thday of study.

Whereas, the lower treatments like 0.1ppm, 1ppm, and 5ppm were rated as moderately damaging during the first half of the week, later all treatments were evaluated as highly damaging except for termites of control that remained acceptable throughout the 2-week study. The condition of termites on day of termination of experiment is given in form of graphs (Fig. 3a). This study is first to perform physical evaluation of termites after treatment with silver nanoparticles.

3.4.2 Physical evaluation of feeder blocks

The physical evaluation of blocks treated with AgNPs remained in very good condition throughout the study. Whereas control wooden blocks were affected by moisture and microbes. Control blocks started to decay very early from the beginning of first week of the study period. Whereas, the condition of treated blocks remained acceptable. During the second week, deterioration started to show in the blocks treated with low concentrations of AgNPs such as 0.1ppm and 5 ppm. Whereas blocks treated with 1ppm, 10ppm and 15ppm remained acceptable. By the end of the study period (two week) blocks treated with 15 ppm of AgNPs remained in best condition, and were not attacked by any microbes. Graphs (Fig. 3b) show the physical evaluation of blocks on the last days of the study. In a similar study effect of nanozincoxide on wooden blocks against microorganism was observed it was effective at 1.2-7.0 ppm (Clausen *et al*., 2011). Silver nanoparticles were effective in protecting the historical objects at 6 mistings with 90 ppm AgNPs for molds, and 8 mistings with 45 ppm AgNPs, two mistings with 90 ppm AgNPs for cotton (Gutarowska *et al*., 2012). In another study, nano-zinc-borate was effective in inhibiting white rot fungus but it could not inhibit brown-rot fungus, Tyromyces palustris explained by Mantanis *et al*. (2014). Similarly nano zinc oxide and nanozinc borate were effective in inhibiting mold fungi whereas nano-CuO and nano-SnO_2 were effective in inhibiting brown rot fungus (Terzi *et al*., 2016). In another study nanometal were effective against white rot fungus as described by Kartal *et al*. (2009). Nanozinc was effective against brown rot fungus as proposed by Németh *et al*. (2013).

3.5 Sensory evaluation of blocks and termites at various AgNPs concentrations

3.5.1 Sensory evaluation of termites

Effect of AgNPs on treated feeder blocks to the termites was evaluated on the bases of sensory parameters. The parameters studied included color, gut appearance and overall appearance of the termites. The study indicated that termites fed to blocks treated with greater concentration of AgNPs were severely effected as compared to those treated by lower concentrations.

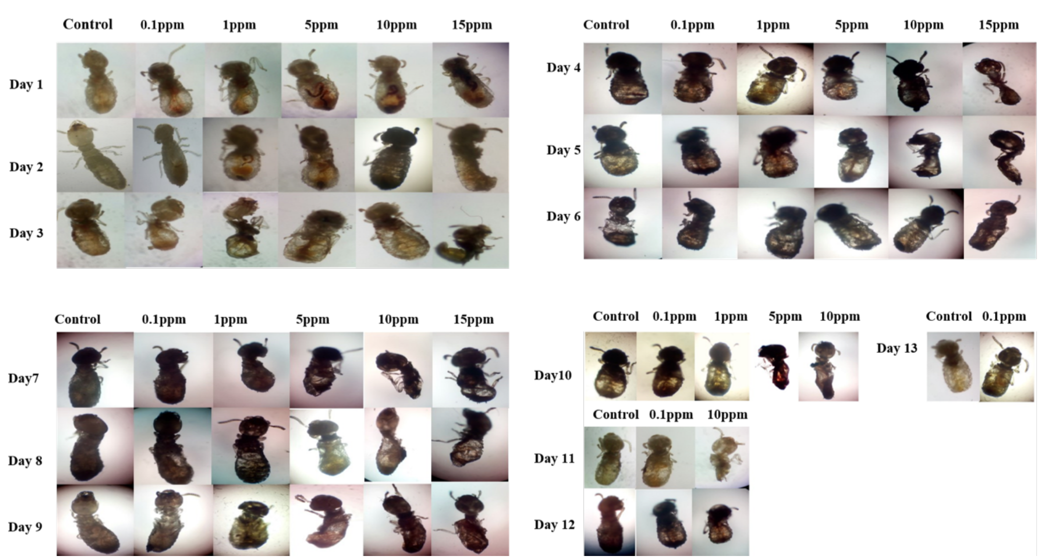


Fig. 4. Physical evaluation of termites. Effect of different concentrations of mycosynthesized silver nanoparticles on termites during the two week study.

The exoskeleton color gradually turned from off white to black. The change was rapid in termites treated with doses of 5ppm, 10ppm and 15 ppm, where change in color occurred within two to three days of exposure. Whereas less change in color occurred in termites fed to blocks treated with low concentrations of AgNPs (Fig. 4). At the end of the experiment color of the exoskeleton of termites of all the treated groups changed except for the termites of control group that remained normal. Effect of AgNPs was also visible on the shape of the termites. Termites fed with blocks that were treated with higher concentration of AgNPs got shrunk from the very first day. This condition was same in almost all treated termites except control that remained unaffected. Prominent damage was visible in the guts of termites fed with blocks treated with AgNPs, whereas the gut of termite of control remained in good condition. Overall appearance of termites of control group remain unchanged while extent of deterioration was prominent with increase in dose (Fig. 4). This study is also first to perform sensory evaluation of termites after treatment with silver nanoparticles.

3.5.2 *Sensory evaluation of feeder blocks*

Termiticidal activity on AgNPs treated feeder blocks was evaluated on the bases of sensory parameters that included observation of color, surface texture, odor and overall appearance. The results showed that blocks treated with 15 ppm of AgNPs were resistant against damage, decay or any fungal attack and were in best condition after two weeks of study. The fastest damaging effect was observed on the control blocks. Followed by blocks of 0.1ppm, 1ppm and 5ppm. The damaging effect became evidently apparent in the second week. Feeder blocks of control started decaying due to fungal growth during the second week and even termites were most active in these blocks. Only control and 0.1ppm blocks were considered as damaged by the end of this study. All other blocks were considered as acceptable (Fig. 5). Studies have shown that metal nanoparticles had played significant roles in protecting wood from termites and other microorganisms as discussed in section 3.4.2.

3.6 *Evaluation of termite damage to treated feeder blocks*

The treated feeder blocks were evaluated in order to observe the damage caused by termites by taking the weight of blocks before and after the two-week study period. The results showed that the blocks treated by higher concentrations suffered minor damage and weight loss whereas the termites suffered major damage.

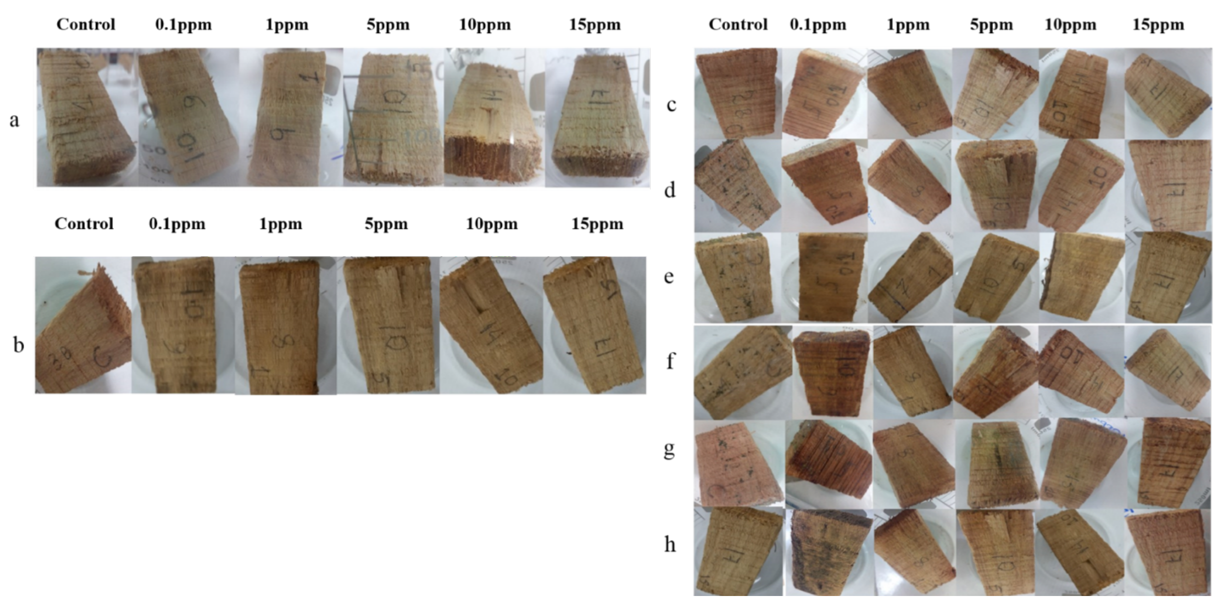
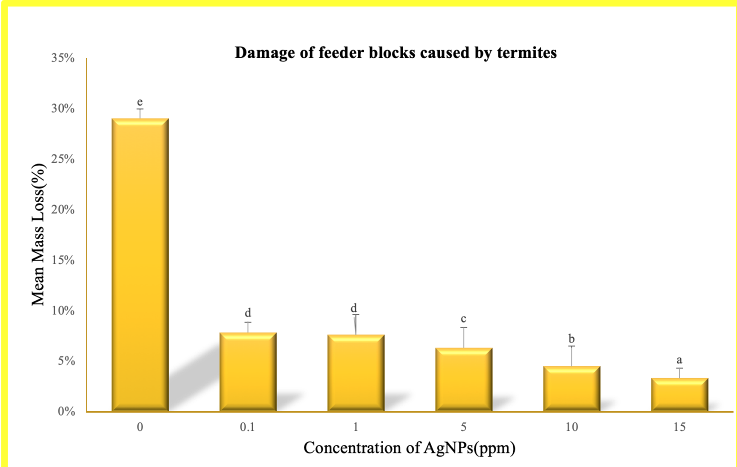


Fig. 5. Condition of feeder blocks with different concentrations of mycosynthesized silver nanoparticles. a) blocks on first day of study week 1 b) blocks on eighth day of study week 2 c) blocks on day 9 d) blocks on day 10 e) blocks on day 11 f) blocks on day 12 g) blocks on day 13 h) blocks on day 14.



Each value is mean of three replicates whereas data labels shows significant difference ($p \geq 0.05$) between treatments analyzed by Duncan multiple range test.

Fig. 6. Evaluation of termite damage to treated feeder blocks. Graph is showing lowest weight loss at 15ppm and highest weight loss in control

The termites of control were in good condition and the feeder blocks underwent major damage including fungal attack. Significant ($p \leq 0.05$) decrease was observed in the weights of 0.0ppm dose which showed mean weight loss was 29% whereas its mortality was 32.2% as compared to 15 ppm dose (Fig. 6). Whereas according to (Shiny *et al.*, 2019) metal nanoparticles were effective in controlling microbial and termite demage to the wood. Another study has reported that nano-zinc-borate was effective enough to inhibit termites and the total mass loss of wood was only 5.2-5.4% (Mantanis *et al.*, 2014). Inclusively mycosynthesized silver nanoparticles were found to be very effective against termites and had efficient termiticidal activity at lower concentrations like 0.1, 1, 5, 10 and 15ppm contrary to the related studies where the metal nanoparticles are applied mostly in greater concentrations ranging from 5000 ppm to 20000 ppm. These silver nanoparticles were also efficient enough in protecting the wood from fungus and mass loss due to termites. Thus, myco-synthesized silver nanoparticles proved an effective termiticide and antifungal agent regarding wood preservation.

Conclusions

Conclusively, mycosynthesized silver nanoparticles has proved an efficient termiticide having potential to inhibit termites at very low concentration within two weeks. Dose of 15 ppm exhibited 100% mortality on 9th day of study, which shows the efficacy of these nanoparticles. Likewise treated wooden blocks were also in a very good condition throughout the study. The percentage mass loss due to termites was also very less in the treated blocks as compared to the control. This shows that these were effective enough in protecting the wood. Further study should be undertaken in order to bring this approach at commercial scale.

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