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African Journal of Microbiology Research

Full Length Research Paper

A comparative study on antibacterial effects of *Hagenia abyssinica* oil extracted from different parts of the plant using different solvents against two selected and standardized human pathogens

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***Hagenia abyssinica* is an important medicinal plant that the traditional society relied on for generations as a traditional medicine for various types of worm infections. The tree is a multipurpose dioecious tree and can grow up to 20 m in length. This research work is designed to investigate the antibacterial activity of *H. abyssinica* oils extracted using three different kinds of solvent and from three different parts of the plant on two standard bacterial isolates obtained from Pasteur Institute located at Addis Ababa, Ethiopia. The solvents used to extract the oils are ethyl acetate, n-hexane and methanol. Accordingly, the antibacterial activity of all the oils were tested and the oil extracted using methanol from all parts of the plants was characterized by having a higher mean of zone of inhibition (which is 1.710 in centimeter diameter) as compared to the two other oils (n-hexane, which is 0910 and that of ethyl acetate, which is 0.842). The data obtained from this research work showed that the oils extracted from the different parts of the plant using the three different solvents exhibited a higher mean zone of inhibition on *Staphylococcus aureus* (which is 1.279) than that of *Escherichia coli* (which is 1.029). In addition, oil extracted from root of the plant has a higher mean of zone of inhibition (which is 1.415) as compared to the leaf (which is 1.14) and bark (which is 0.908). Generally, oils extracted from root, leaf and bark of *H. abyssinica* have an antibacterial property even if they exhibit difference.**

Key words: *Hagenia abyssinica,* oil, solvents, antibacterial activity, zone of inhibition, *S. aureus, E. coli.*

INTRODUCTION

Medicinal plants have been used since antiquity to treat various health problems, and about 80% of the Ethiopian people rely on traditional medicine to meet their healthcare needs (Endashaw, 2007). The widespread use of traditional medicine could be attributed to cultural acceptability, perceived efficacy against certain types of diseases, physical accessibility and affordability as compared to modern medicine (Girma, 1998; Debela et

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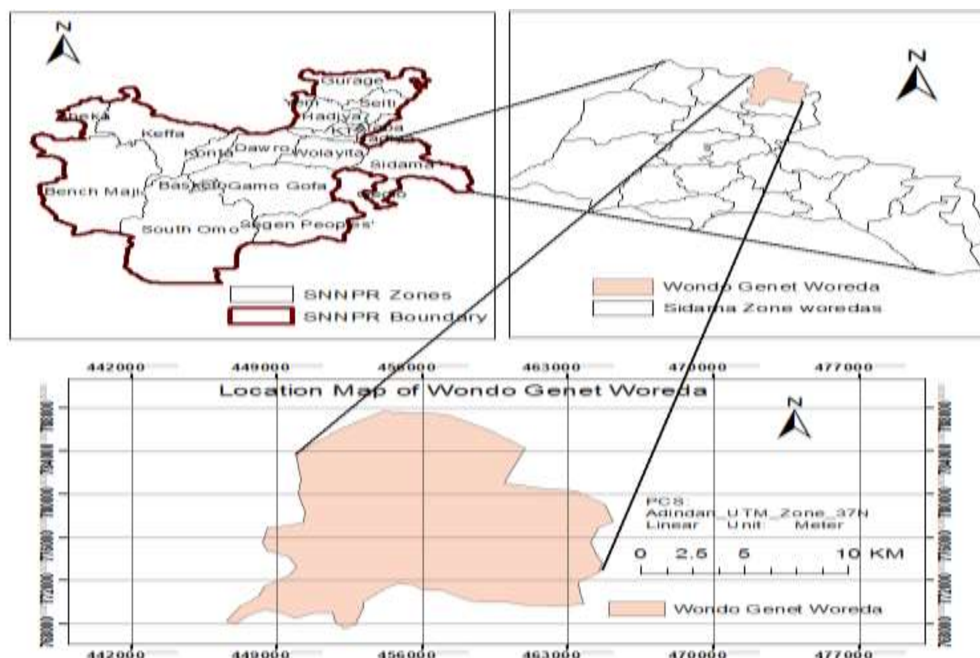

Figure 1. Map showing the location of the study area.

al., 2006; Endashaw, 2007).

Hagenia abyssinica is one of the key medicinal plants which people relied upon for generations to get-rid of various ailments. The species was once abundant in the semi-humid mountain woodlands of Ethiopia with altitudinal range between 2450 and 3250 m above sea level (Hedberg, 1989), but now the species is endangered and sparsely distributed due to over exploitation (Legesse, 1995). *H.abyssinica* is a multi-purpose tree that has every part of it used for different purposes such as medicine, timber, firewood, poles, mulch, green manure, and as an ornamental plant. The species has been widely used for its potent anti-tapeworm activity (Tileye, 2006; Biruktayet et al., 2010). Despite its endangered state and high call for conservation, detailed information on the agronomic and chemical traits of the species is lacking. Proper utilization and conservation efforts have not been established to take all the necessary actions to rescue this species.

Previous ethno-botanical studies on medicinal plants focused on the free listing of traditional medicinal plant species. To this end, medicinal plant, shrubs and trees, which are currently getting acceptance in herbal medicines of the country and also possessing higher genetic endemism such as *H. abyssinica* need to be considered for pharmacological analysis. The purpose of the present study is, therefore, to investigate the antibacterial effect of extracts of *H. abyssinica* on human pathogens.

The general objective of the study was to conduct a comparative study on the antibacterial effects of *H. abyssinica* oil extracted from different parts of the plant using different solvents against two selected and standardized human pathogens. The oils extracted from the different parts of the plant using the three different solvents were assessed for their antibacterial activity of against two selected and standardized human pathogens namely *Staphylococcus aureus* ATCC 25923 and *Escherchia coli* ATCC 25922. In addition, the research work has an aim of identifying which parts of *H. abyssinica is* most effective against the activity *of S. aureus* ATCC 25923 and *E. coli* ATCC 25922 and also intended to identify the effective solvent used for oil extraction from different parts of *H. abyssicnica* on the antibacterial action of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

MATERIALS AND METHODS

Study area

Wondo Genet Area is located in Wondo Genet Woreda, Southern Nations Nationalities and Peoples Regional State. This area is located 265 km away from Addis Ababa, the capital of Ethiopia. It is part of the Sidama Zone, located in the Great Rift Valley and is bordered on the south by Malga, on the west by Awasa Zuria, and on the north and east by the Oromia Region. The Woreda is located between 5°30′ N to 7° 20′ N latitude and 37° 05’ E to 39° 50’ E longitude and the elevation ranges from 1680 to 3960 m above sea level (Bizuwerk, 2004). The *H. abyssinica* plant samples were collected from the field and brought to the laboratory for extraction (Figure 1).

The data collection for this experimental research work consists of two major experimental steps. The first was the extraction of the crude oil from the different parts of the plant using different solvents namely methanol, ethyl acetate and n-hexane. The second

experimental step was testing the oils extracted from different parts of the plant using different solvents in response to the two selected reference species of bacteria namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

The experimental work manipulates three factors namely *H. abyssinica* parts (root, leaf and bark), different alcohol solvent (methanol, ethyl acetate and n-hexane) and two different species of standardized pathogenic bacteria species (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922). The first two factors had three treatments and the third contains two species of standardized bacteria. The experiment has a total of eighty (18) treatment combinations each replicated four times to test the extent of growth inhibition on the two bacterial species namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Hagenia abyssinica sample collection and oil extraction method

Healthy looking root, leaf and bark sample of *H. abyssinica was* collected from Wondo Genet College of Forestry and Natural Resources and transported to the laboratory ascetically. The bark and leaf parts were taken from medium position of the plant height whereas the root was taken from the lateral roots possibly because the traditional medicinal plant hunters used the matured parts of the plant for its medicinal purpose. The solvents used for extracting the oil were n-hexane, ethyle acetate and methanol (Hesham et al., 2016; Akinyemi et al., 2006).

The plant materials were collected; the fresh weight was recorded and dried using an oven dry method at the temperature of 40°C for three days. Thereafter, the fresh and dry weights of the plant part were measured using a digital balance. The dried sample was then crashed into smaller pieces using a clean laboratory cutting mill machine. Afterwards, 30 g of the dried sample was taken from the crushed and dried (leaf, root and bark) parts of the plant, was placed in a plastic bottle and these were diluted with 180 mL of ethyl acetate, n-Hexane and methanol. The bottles containing the solvents and dried sample were placed on an electrical mechanical shaker at 200 rpm. The solution was stayed on mechanical shaker for three successive days (Hesham et al., 2016; Akinyemi et al., 2006).

After three consecutive days of shaking, the solution was separated from the shaker and the residue was filtered with Whitman Filter Paper No 1. Then the liquid containing both the solvents and extract was placed in an oven drier adjusted at 30°C. In order to separate the solvent from the crude plant extract the solution was placed inside an oven drier for four consecutive days. After evaporation of the solvents from the crude oil extracts, the effectiveness of the crude oil extracted against bacteria types was tested according to the procedure of Hesham et al. (2016) and Akinyemi et al. (2006).

Test microorganisms and Inoculum preparation

Microorganisms used in the experiment are standard reference species with the American Type Culture Collection (ATCC) (*S. aureas* ATCC 25923 and *E. coli* ATCC 25922) obtained from Pasture Institute Laboratory located at Addis Ababa, Ethiopia. A Muller Hinton, McConkey and Nutrient agar were prepared and autoclaved at 121°C, 15 psi for 20 min. Thereafter, the autoclaved medias were placed inside a clean laminar flow cabinet, allowed to cool to 45°C, poured aseptically into sterilized petriplates and the plates were stayed overnight inside a clean laminar flow cabinet. The reference bacterial species which are stored in the Muller Hinton broth were aseptically inoculated using a spread plate method on the media by using a sterilized bent glass rod (Coyle, 2005; Akinyemi et al., 2006).

Antibiotic assay

The antibiotics diffusion method (Singh et al., 2006) was used to evaluate the antibacterial activity of the crude oils extracted using the three solvents. To activate growth, the standard reference species were cultured overnight at 37°C on Muller Hinton Broth and Nutrient Broth and the study organisms were inoculated on Nutrient agar and McConkey agar media. Sterilized discs of equal area were prepared using a clean cork borer and the discs were inoculated inside the extracted oils for more than three days until the discs absorbed the oil sufficiently in the different oils extracted from root, leaf and bark of the plant using different solvents in separate inside beakers (Akinyemi et al., 2006).

Four discs per plate from oil of each plant parts extracted using different solvents were inoculated on the media containing the standard reference species; the plates were then sealed with a parafilm and incubated at 37°C for 72 h. After incubation for three days, the plates were observed for the presence of clear inhibition zone around the discs. For each treatment, a replication was made and the zone of inhibition was measured in centimeters (Akinyemi et al., 2006).

Data analysis

After the necessary data collection was made, the data collected were organized and analyzed using SPSS version 16 and the findings were presented using tables and graphs. The means of zone of inhibition for the different oils extracted from the different plant parts using different solvents were compared using LSD test. Significant differences of the three factors were tested at α of 0.05. Finally, the result was presented in the form of tables, percentages and graphs.

RESULTS AND DISCUSSION

Effect of plant crude oil extract on bacterial growth

H. abyssinica is a plant commonly found in high altitude areas in East Africa. It is an important medicinal plant with the flower reported as antihypertensive, taenicidal (Desta, 1995), antihelminthic, trypanocidal (Nibert and Wink, 2010), and in treatment of eye disease (Abebe and Ayehu, 1993). *H. abyssinica* is a plant commonly found in high altitude areas in East Africa. It is an important medicinal plant with the flower reported as antihypertensive, taenicidal, (Desta, 1995), antihelminthic, trypanocidal (Nibert and Wink, 2010), and in treatment of eye disease (Abebe and Ayehu, 1993).

H. abyssinica is a medicinal plant commonly found in the highlands of East Africa and characterized by its medicinal property for various type of bacterial and worm infection by the local community for centuries (Lilian, 2013; Biruktayet et al., 2010). It has been serving the traditional community as an antihelmintic in ruminants and also against tapeworms in humans (Biruktayet et al., 2010); even the local name of the tree 'koso' refers to the tapeworm infecting human being. Wolde et al. (2016) has reported that the chemicals found inside the crude oil extract of the plant have an antibacterial property and the antibacterial compounds found inside the oils either kill or inhibit the growth of the inoculants. Accordingly, the crude oils extracted using the three different solvent and

Table 1. Mean zone of inhibition (± Standard Error) of the oils from different parts of the plant on *S. aureus* and *E. coli*.

Parts of the plant	Mean ± Std. Error
Root	1.415[a] ± 0.069
Leaf	1.140[b] ± 0.069
Bark	0.908[c] ± 0.069

Table 2. Mean zone of inhibition (± Standard Error) of the oils extracted using different solvent from different parts of *H. abyssinica* on the test organisms.

Solvent type	Mean ± Std. Error
Ethyl acetate	0.842 b ± 0.069
Hexane	0.910 b ± 0.069
Methanol	1.710 a ± 0.069

Table 3. Growth inhibition response of bacteria types for the extract of solvent and parts of *H. abyssinica*.

Bacteria type	Mean ± Std. Error
Staphylococcus aureus	1.279 a ± .057
Escherichia coli	1.029 b ± .057

from the different organ of the plant shows that they have an antibacterial activity even if their strength differ between solvents and parts of the plant. This is highly supported by the finding of Wolde et al. (2016) who stated that the presence of saponins, phenols and alkaloids could confer antibiotic property of the plant (Wolde et al., 2016).

The current study indicated that there is a considerable difference in the antibacterial activity of the oils extracted from different parts of the plant and extracted using different solvents (ethyl acetate, n-hexane and methanol). The oils extracted from root and leaf of *H. abyssinica* was more active in their antibacterial activity than the oil extracted from bark (Table 1). In addition to this, the oils extracted using methanol has a significant antibacterial activity than the oils extracted using ethyl acetate and n-Hexane (Table 1). This might be due to the high crude yield extract obtained using methanol as compared to ethyl acetate and n-hexane (Wolde et al., 2016).

Accordingly, besides interaction of parts of *H. abyssinica* with the selected bacterial species, the other three factors (solvent types, bacteria species types, parts of *H. abyssinica*) and the interactions (Plant Parts × Solvent types, Solvent × Bacteria types, and Plant Parts × solvent types × Bacteria types) were significantly different at $\alpha < 0.05$ (Tables 1, 2 and 3).

As shown in Table 1, the different parts of the study plant have different mean zone of inhibition (measured in centimeters) of bacterial growth. The root part of the study plant was characterized by having high zone of inhibition as compared to other parts of the plant and it is statistically significant at $\alpha = 0.05$ which is followed by leaf and bark.

The mean zone of inhibition while comparing the solvents showed that there is a difference with regard to inhibition of growth of the two selected species of bacteria. The oil extracted using methanol as a solvent resulted in higher mean zone inhibition (1.710) followed by n-hexane (0.910) and ethyl acetate (0.842) respectively on both species of bacterial isolates (Table 2).

The test on the two bacterial species showed that the two different bacteria had different mean of resistance to the extracted oil from the different parts of the plant. *S. aureus* result indicating higher mean of inhibition shows that it is relatively sensitive to the oils or have high inhibition zone as compared to *E. coli* (Table 3). This finding is in accordance with that of Wolde et al. (2016) which revealed that the largest zones of inhibition were recorded with methanol crude extract from *H. abyssinica* against *S. aureus.*

Interaction effect of solvents, parts of *H. abyssinica,* and bacteria types on the growth inhibition

As indicated in Figure 2, oil extracted from the root part of *H. abyssinica* using methanol as a solvent inhibit the growth of the two bacterial species better than that of leaf and bark extracts by the three solvent types (Ngeny et al., 2013). Oil extracted from bark part using hexane solvent resulted in lower mean zone of inhibition. This finding was also supported by Wolde et al. (2016) who stated that the antibacterial activity of methanol crude extract was better than ethanol extracts, and hexane.

When the means for the interaction effects for parts of the plant was carefully observed, the highest zone of inhibition was found on *S. aureus* than *E. coli* (Figure 3), indicating that *S. aureus* is highly sensitive to the crude oil extracts of *H. abyssinica* (Wolde et al., 2016, Ngeny et al., 2013). The root of *H. abyssinica* was recorded as having the highest zone of inhibition which was followed by the leaf and bark.

The study shows that the highest zone of inhibition from the interaction effect of the solvents and bacterial species was recorded for those oils extracted using methanol (1.725 for *S. aureus* and 1.696 for *E. coli*) followed by those oils extracted using hexane and ethyl acetate (Figure 4). When the relative mean of zone of inhibition in all the three solvents were compared, the highest mean of zone of inhibition was recorded on *S. aureus*. Similar finding by Wolde et al. (2016) and Ngeny et al. (2013) reported that *S. aureus* is sensitive to *H. abyssinica* crude oil extract.

The highest mean zone of inhibition by the oil extracted from root was recorded for the one which was extracted using methanol as a solvent (Figure 5). Similarly, the highest mean zone of inhibition by the oil extracted from

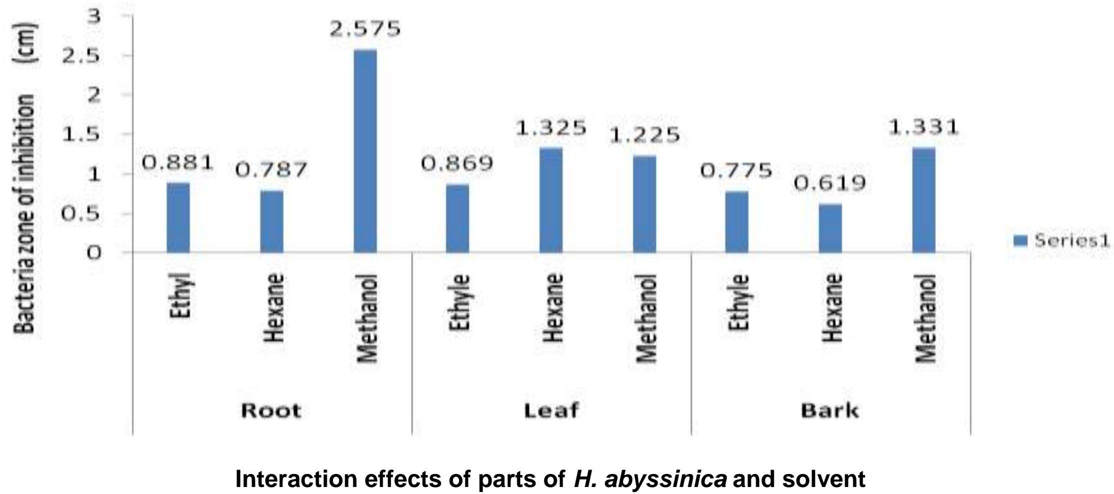

Figure 2. Interaction effects of parts of *H. abyssinica* and solvent types.

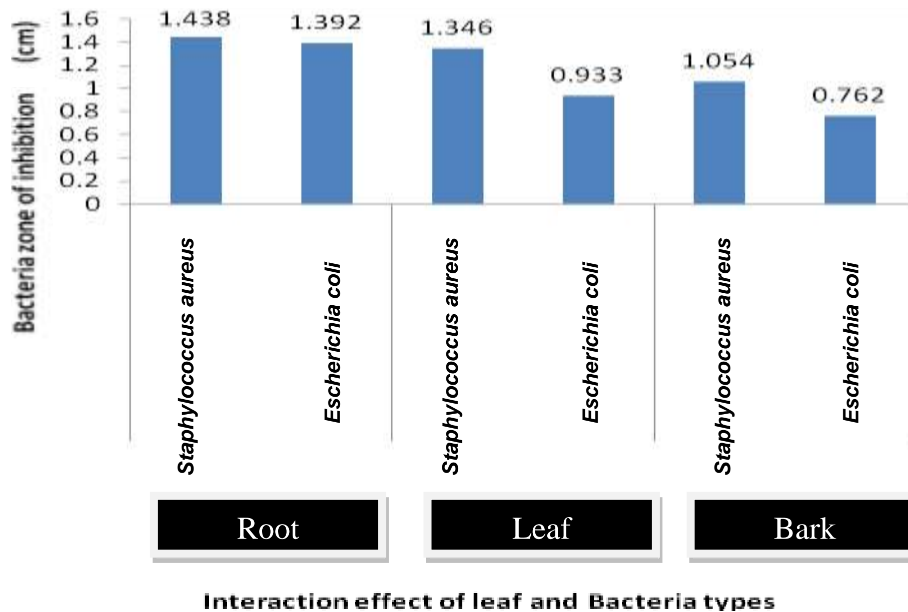

Figure 3. Interaction effects of parts of *H. abyssinica* on *S. aureus* and *E. coli*.

bark was recorded for the one which was extracted using methanol as a solvent. This might be the relatively higher crude oil extract gained by using methanol as a solvent, with similar finding also reported by Wolde et al. (2016) and Ngeny et al. (2013). However, the highest mean zone of inhibition by the oil extracted from leaf was recorded for the extract using hexane. In addition, the highest mean zone of inhibition was recorded on *S. aureus* (2.438) and *E. coli* (2.713) by the oil extracted using methanol and the root part of the plant followed by the oil extracted using methanol from bark of the plant (1.537 and 1.125, respectively) (Figure 5).

Conclusions

It was observed from this research work that oils extracted (using different solvents) from different parts (leaf, stem and bark) of *H. abyssinica* oils exhibited antibacterial property on *S. aureus* and *E.coli* with great difference in the diameter for zone of inhibition measured in centimeter. The oils extracted from the different parts of the plant has an antibacterial activity on the two selected study microorganisms even if there is difference in their strength between parts and between solvents.

While comparing the effectiveness of the oils extracted

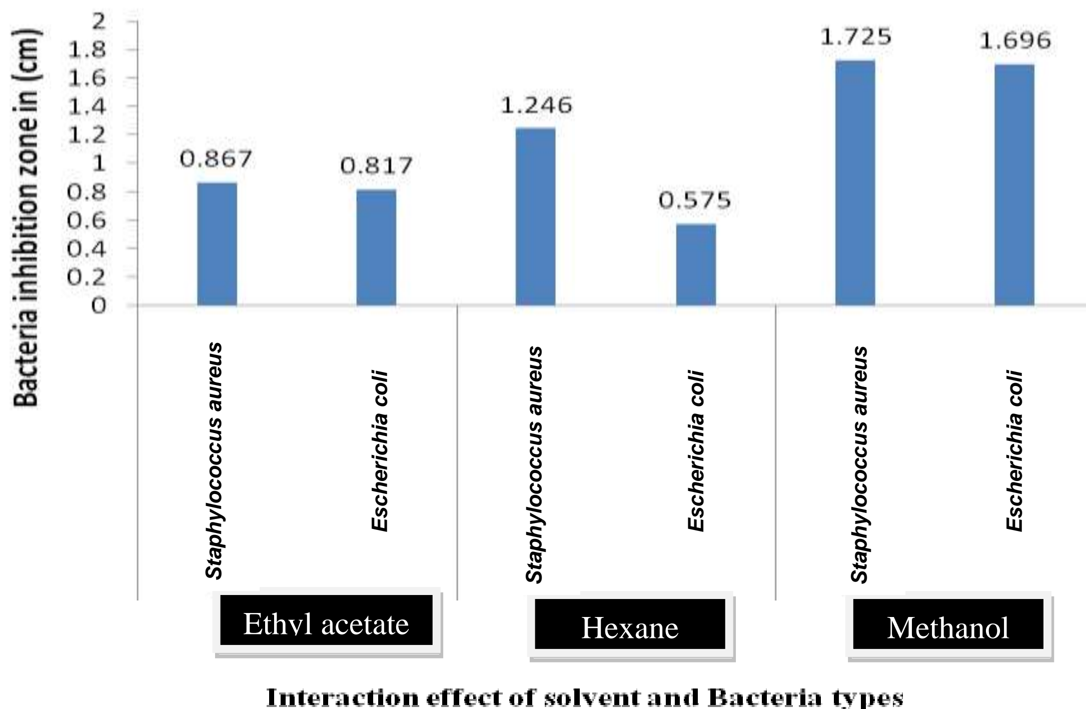

Figure 4. Interaction effects of solvent and Standardized Bacterial species types.

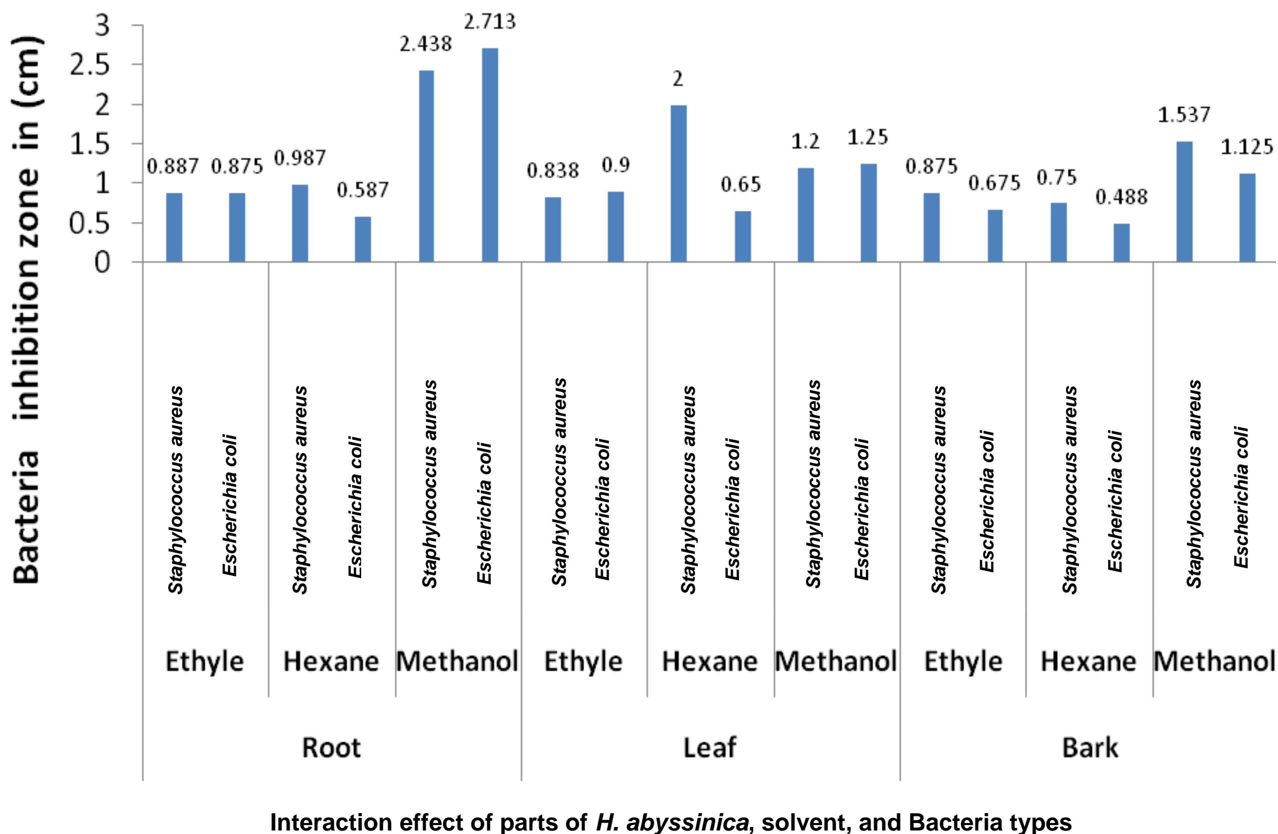

Figure 5. Interaction effects of parts of *H. abyssinica*, solvent, and bacteria types on growth inhibition zone.

using the different types of solvents, methanol exhibited relatively higher antibacterial activity on the selected study microorganisms followed by ethyl acetate and n-hexane. The oils extracted from root of *H. abyssinica* with all the three solvents have a better antibacterial activity as compared to other parts of the plant.

The oil extracts of *H. abyssinica* has a relatively better inhibitory effect on *S. aureus* than that of *E.coli.* The extracts of *H. abyssinica* from the three different parts were found active on both of the selected standard species of bacteria. This indicates that the oils have either cytotoxic or inhibitory effect and further investigations are therefore needed to clarify which compound is responsible for cytotoxic or inhibitory activities of the oils.

RECOMMENDATIONS

Data obtained from this research work put forward the following recommendations:

i) Chemical and pharmaceutical industries should work together with researchers and traditional medicinal plant collectors in order to utilize the maximum benefit of the plant under study.
ii) It is now understood in this research work that *H. abyssinica* oil has a remarkable cytotoxic effect on bacteria and further study is needed to indicate which compound inside the oils is responsible for the antibacterial property of the oil.
iii) Further researches should focus on the investigation of the antibacterial property of *H. abyssinica* parts and the minimum inhibitory concentration showing anti-bacterial activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Biosynthesis of cadmium selenide quantum dots by *Providencia vermicola*

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Cadmium selenide quantum dots (CdSe QDs) were synthesized by using *Providencia vermicola* BGRW, which has discriminatory ability to resist many metals such as Selenium, Cadmium, Silver, Zinc, Copper, Lead, Nickel, Cobalt and Bismuth metals. BGRW was able to grow in the presence of 6 mM of $CdCl_2$. The best conditions for extra/intracellular CdSe QDs synthesis were 0.1 mM SeO_2: 0.9 mM $CdCl_2$, 37°C, pH 9 within 24 h. The biosynthesis of CdSe QDs was monitored by UV–Visible spectrum that showed surface plasmon resonance (SPR) peaks at 388 nm. Depending on fluorescence property of quantum dots, photoluminescence characteristics of the biogenic CdSe QDs were at 385 nm. Further characterization of synthesized CdSe QDs was carried out using the X-ray Diffraction (XRD), Transmission Electron Microscope (TEM) and Fourier Transform Infrared (FTIR) spectroscopy. TEM and XRD analysis revealed that CdSe QDs was cubic in shape with a size range of ~2 to 4 nm. EDS analysis confirmed the composition of QDs from cadmium and selenium ions. FTIR spectroscopy confirmed the presence of proteins as the stabilizing agent surrounding the quantum dots.

Key words: Quantum dots, cadmium selenide quantum dots, capping agent, transmission electron microscope, fluorescence spectroscopy, X-ray and FTIR analysis.

INTRODUCTION

Quantum dots (QDs) are 1-10 nm semiconductor nanoparticles that possess size-dependent luminescence, the QDs usually refer to the II−VI, III−V and IV−VI binary and their alloyed semiconductor materials with a confined size in three dimensions (Kumar et al., 2007). The QDs have attracted tremendous attention from materials scientists, physicists, chemists and biologists worldwide due to a number of distinguished characteristics, including prominent optical performance with good photo-stability (Dubertret et al., 2002; Wu et al., 2003; Medintz et al., 2005), high quantum yield (QY) and long fluorescence lifetime (Medintz et al., 2005). Also included are simultaneous excitation of multiple QDs by a single light source (Han et al., 2001), narrow, symmetrical and size-tunable emission spectra coupled with wide absorption spectra (Chan et al., 2002) and broad spectral windows spanning from the ultraviolet to infrared region (Medintz et al., 2005).

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One of the QDs, cadmium selenide (CdSe), attracts awareness because it is beneficial for light emitting diodes, solar cells and biological imaging (Bera et al., 2010). Previously, CdSe QDs were produced, using organometallic chemical techniques (Danek et al., 1994; Alivisatos, 1996). Furthermore, numerous efforts have been initiated to investigate new capping structures such as dodecyl amine and stearic acid for CdSe (Hines and Guyot-Sionnest, 1996; Peng et al., 2000; Peng and Peng, 2001). Notably, some bacteria exhibit a strong reducing capability toward the semiconductor precursors (Malarkodi et al., 2014). The revelation of natural metal-related bio-detoxification, bio-mineralization and enzymatic reactions really facilitates the understanding of QDs formation mechanisms in biological systems (Dameron and Winge, 1990; Crookes-Goodson et al., 2008; Saa et al., 2010). *Fusarium oxysporum* in a mixture of $CdCl_2$ and $SeCl_4$ synthesized CdSe nanoparticles (9-15 nm) (Kumar et al., 2007). Also, CdSe QDs were synthesized using *Saccharomyces cerevisiae*, which is widely used as a model organism and an ideal candidate biosynthesizer for fluorescent semiconductor CdSe QDs (Cui et al., 2009). In addition, Pearce et al. (2008) synthesized CdSe QDs by adding $CdCl_2O_8$ to selenide produced from selenite by *Veillonella atypica,* which is an anaerobic bacterium. In two latter studies (Pearce et al., 2008; Cui et al., 2009), cadmium was added after microbial formation of selenide for CdSe production in two-vessel processes, likely for the Cd toxicity to the microbes. In contrast, Kumar et al. (2007) showed a one-vessel process in which the fungus produces CdSe in the co-presence of selenite and cadmium ion, which might boost economic efficiency through its simple operation of fewer reaction vessels and culturing without microbial contamination. Similarity, Ayano et al. (2014) isolated *Pseudomonas aeruginosa* strain RB from a soil sample, which has capacities of both selenite reduction and cadmium resistance. This bacterium can synthesize 10-20 nm CdSe particles inside and outside the cells from selenite and Cd ion by a one-vessel reaction particle larger than 100 nm were noticed outside the cells. The value of the atomic Se/Cd ratio of the produced particles was 1.0, which was the ideal value (Ayano et al., 2014). The CdSe produce would compete with the CdS synthesis under alkaline state, resulting in high S contents and low Se/Cd ratios compared with other conditions (Ayano et al., 2015). Actually, CdSe is known to appear reddish brown (Kumar et al., 2007), whereas SeNPs and CdS show intense red (Ayano et al., 2014) and bright yellow (Ahmad et al., 2002), respectively.

QDs have interest in many industrial research applications such as transistors, computing, photovoltaic devices, photodetector devices, photo-catalysts, LEDs, diode lasers and second-harmonic generation because of the high tenability of properties (Ramirez et al., 2015). To benefit from the interdisciplinary research among nanotechnology, chemical processing, biotechnology and systems engineering tremendous progress has been made in the biomedical applications of QDs during the past two decades since the first study regarding their biological applications were reported in 1998 (Bruchez et al., 1998). QDs are an important class of fluorescent labels with prominent optical features and can be combined with advanced biological and imaging techniques for molecular and cellular imaging (Zhou et al., 2015). Additionally, the QDs have large surface areas and may function as effective nano-scaffolds to conjugate miscellaneous probes for ultrasensitive bioassays and diagnostic applications (Kairdolf et al., 2013). BGRW strain of *Providencia vermicola* has a discriminatory ability to produce selenium nanoparticles, resist many metals such as selenium, cadmium, silver, zinc, copper, lead, nickel, cobalt and bismuth metals and able to grow in the presence of 6 mM of $CdCl_2$ (El-Deeb et al., 2018). Therefore, this study investigated effects of culture conditions such as pH, temperature and Se/Cd ratios on the rate of CdSe synthesis of the strain BGRW, which have ability to produce CdSe QDs isolated from a rhizosphere in the farm at Taif, KSA.

MATERIALS AND METHODS

Bacterial strain

Strain BGRW was used for this study; this strain was revealed to synthesize selenium nanoparticles (El-Deeb et al., 2018). The 16s rRNA sequence of this strain was reported later by the previous study as accession KX447430 in the GenBank database.

Reduction of cadmium and selenium ions

Previously isolated strain BGRW as a selenium reducing bacteria was screened for selenium-cadmium reducing ability. Bacterial strain was grown on tryptic soy agar (TSA) supplemented with 0.5 mM selenium dioxide and 0.5 mM cadmium chloride at 37°C for 24 h according to Ayano et al. (2015) and Cui et al. (2009) with slight modification. If colonies just grow, a strain is classified as resistant but if the colonies take yellow-orange color, a strain is classified as cadmium and selenium reduced strain.

Determination of minimum inhibitory concentration (MIC) of SeO_2 and $CdCl_2$

The metal salts SeO_2 and $CdCl_2$ were used in varying concentrations (0.5-25 mM) supplemented onto a nutrient agar plate. The metal resistant bacterial colony was scored and sub-cultured on tryptic soy agar (TSA) with different concentrations of various metal salts such as SeO_2 and $CdCl_2$and incubated at 37°C for 24 h. The MIC is expressed as the minimum concentration of various metal ions that completely inhibited the appearance of colonies on the plates. Experiments were carried out in triplicate (Ghosh et al*.,* 2008).

Ability of BGRW to resist various metals

Tryptic soy agar amended with 1 mM of Silver, Zinc, Copper, Gold, Lead, Nickel, Cobalt and Bismuth were added to the medium as $ZnCl_2$, $PbCl_2$, $CuCl_2$, $AuCl_3$, $AgNo_3$, $BiCl_3$, $NiCl_2$ and $CoCl_2$ (Piotrowska-Seget et al., 2005). Each of these metals was added in

a separate experiment. The plates were incubated at 37°C for 48 h. Metals resistance is the ability of microorganism to resist killing by used metal.

CdSe QDs synthesis, collection and purification

The isolated bacteria were cultivated aerobically in 100 mL tryptic soy medium supplemented with 0.1 mM SeO_2 and 0.9 mM $CdCl_2$ (the best ratio which determined later) in 250 mL flasks in one-vessel production of QDs. This is so since SeO_2 and $CdCl_2$ salts were added in one step on a rotary shaker (110 rpm) for 24 h at 37°C in the dark (Ayano et al., 2015). Formation of CdSe quantum dots was monitored, by recording the absorbance spectrum (200–800 nm) at regular intervals.

QDs collection was different, depending on their location when cells containing the particles as intracellular production bacteria were collected by centrifugation (7000 rpm, 4°C, 10 min) from 50 mL of the culture and washed three times with ultrapure water. Subsequently, the pellet was resuspended in 5 mL of Phosphate Buffer solution (pH 7.2) as a wash buffer and followed by cell-disruption with an ultra-sonicator (130 W, 10 min, Vibracell VCX-130; Sonics and Materials Inc., CT, SA); the suspensions were filtered with 0.25 µl Millipore syringe filters. Then the QDs were collected from the filtrates by centrifugation (10,000 rpm, 4°C, 30 min) and suspended in 5 mL of ultrapure water according to Cui et al. (2009) with slight modification. In addition, another easy procedure of QDs biorecovery was used, whereas the cells containing QDs were sterilized at ordinary autoclaving program according to Fesharaki et al. (2010) with slight modification.

But when the production of QDs was extracellular, bacteria were precipitated using centrifugation (7,000 rpm, 4°C, 10 min) from 50 mL of the culture then the supernatant was filtered through 0.25 µl Millipore syringe filters to obtain free cell supernatant which had free QDs. Then, the QDs were collected using centrifugation (10, 000 rpm, 4°C, 30 min) and suspended in 5 mL of ultrapure water (Suresh, 2014). The precipitate of QDs from intra/extra cells was washed with ethanol and water to remove any contaminants present and dried in hot air oven at 45 -50°C; this step is called purification (Shivashankarappa and Sanjay, 2015). The CdSe QDs were purified from the protein and other contaminants.

Characterization of CdSe QDs

Fluorescence or UV-Vis spectrophotometer

The characterizations of the synthesized QDs were carried out according to the method described previously (Ahmad et al., 2003). The biologically synthesized CdSe QDs were characterized by UV-Vis spectroscopy (Perkin Elmer, Lambda 25) instrument scanning in the range of 200-800 nm, at a resolution of 1 nm. However, the fluorescence excitation spectra of all CdSe QDs samples were measured with a fluorescence spectrophotometer at an excitation wavelength of 380 nm (RF-5301, Shimadzu; Ex = 380 nm), using the same settings. All samples for fluorescence or UV-Vis spectra measurement analysis were conducted to collect and purify CdSe QDs as mentioned above at 25°C; all samples were diluted 10-fold for all experiments. UV-Vis spectroscopy analyses of cell-free supernatant without the addition of SeO_2or $CdCl_2$ was used as control throughout the experiment. Analysis of several weeks old samples was also carried out to check the stability of CdSe QDs.

Transmission electron microscopy (TEM) measurements

The sample was first centrifuged at 10,000 rpm for 30 min. For transmission electron microscope (TEM) measurements, a drop of solution containing synthesized CdSe QDs was placed on the carbon coated copper grids and kept under vacuum desiccation for overnight before loading them onto a specimen holder. Studies of size and morphology of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 k accelerating voltage (JTEM 1230, Japan, JEOL) at King Saud University.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was performed using an automated diffract meter (Philips type: Pw1840), at a step size of 0.02, scanning rate of 20 in 2θ /min, and a 2θ range from 10 to 80. Indexing of the powder patterns and least squares fitting of the unit cell parameters was
possible using the software X'Pert High score Plus.

Electron Dispersive Spectrometer (EDS) analysis

The chemical elements that form the purified quantum dots were determined with EDS. For that purpose, recovered pellets of CdSe QDs were analyzed with an energy-dispersive X-ray spectrometer (Hitachi S-570, Tokyo, Japan).

Fourier Transforms Infrared Spectroscopy (FTIR) analysis

FTIR measurements were carried out using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrometer (Bruker, Germany, Alpha-P). The instrument was configured with ATR sample cell including a diamond crystal with a scanning depth up to 2 µm. To remove any free biomass residue or compound that is not capping ligand of the QDs, the residual solution of 100 ml after reaction was centrifuged at 10,000 rpm for 30 min. The bio transformed products present in cell-free filtrate, freeze-dried sample powders, were applied to the surface of the crystal then locked in place with a "clutch-type" lever before measuring transmittance. Each of the spectra was collected in the range 400- 4,000 cm^{-1} at 2 cm^{-1} resolution. Compared to the conventional transmission mode, the present technique is faster sampling without preparation, excellent reproducibility and simpler to use.

Optimization of CdSe QDs biosynthesis

The effect of temperature on CdSe QDs formation

All assays to investigate the effect of various parameter on the reduction ability by inoculated pure microbial cultures in 250 ml Erlenmeyer flasks containing 100 ml tryptic soy broth (Ayano et al., 2015). In addition to the required volume of 200 mM stock solutions of SeO_2 and $CdCl_2$ was done to give an overall concentration of 0.1 mM SeO_2: 0.9 mM $CdCl_2$, which was the best ratio to the biosynthesis of CdSe QDs and the pH of the aqueous mixture was adjusted to pH 7.0. The effect of temperature on CdSe QDs formation was evaluated with assay by incubating the samples at a temperature range between 10 and 60°C. The flasks were incubated on a shaker at 110 rpm for 24 h, each temperature was carried out in duplicate; no inoculum was added to sterile controls. The synthesis of CdSe QDs was followed by visual observation of orange-yellow color formed in the culture; UV-Vis spectrums were also obtained.

The effect of SeO_2 and $CdCl_2$ concentration on CdSe QDs formation

This was done by the inoculation pure microbial cultures in 250 ml Erlenmeyer flasks containing 100 ml tryptic soy broth with different

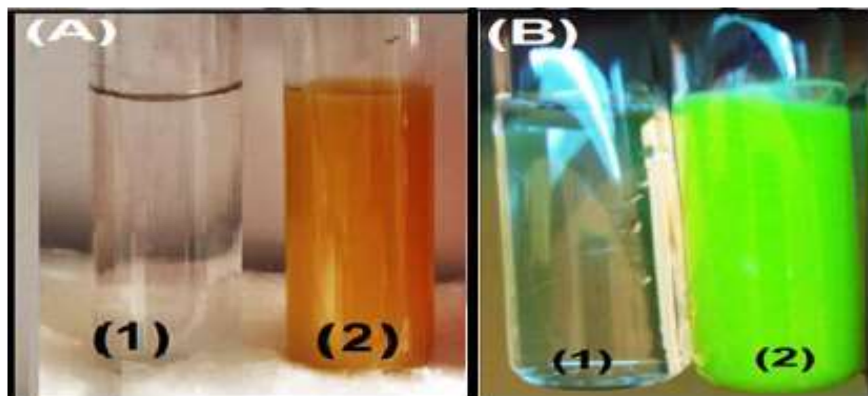

Figure 1. (A) A photograph showing orange-yellow CdSe QDs in a tube (2) and no color change at negative control in a tube (1) and (B) a photograph showing the previous tubes under UV-lamp, fluorescence property of CdSe QDs appear clearly in a tube (2).

ratios of SeO_2 and $CdCl_2$ in the assays (0.1 mM: 0.9 mM, 0.2 mM:0.8 mM, 0.3 mM:0.7 mM, 0.4 mM:0.6 mM, 0.5 mM:0.5 mM, 0.6 mM:0.4 mM, 0.7 mM:0.3 mM, 0.8 mM:0.2 mM and 0.9 mM:0.1 mM). All these concentrations were obtained by adding the required volume of 200 mM stock solutions of SeO_2 and $CdCl_2$. Each ratio was carried out in duplicate; no inoculum was added to sterile controls. The flasks were incubated on a shaker at 110 rpm and 37°C for 24 h according to Suresh et al. (2014) with slight modification. The synthesis of QDs was followed by visually observation of gradation color from red to orange-yellow formed in the culture, UV-Vis and fluorescence spectrums were also obtained as previously described (Wu et al*.,* 2015).

The effect of pH on CdSe QDs formation

All assays to investigate the effect of pH on the reduction ability by inoculated pure microbial cultures in 250 ml Erlenmeyer flasks containing 100 ml tryptic soy broth were done (Ayano et al., 2015). Adding to the required volume of 200 mM stock solutions of SeO_2 and $CdCl_2$ to give an overall concentration of 0.1 mM SeO_2: 0.9 mM $CdCl_2$ was done. This was the best ratio to biosynthesis of CdSe QDs. The effect of pH on CdSe QDs formation was evaluated using specific buffers, ranging from pH 5 to 9. Acetate was used for the acidic pH range and NaOH for the alkaline pH range. The flasks were incubated on a shaker at 110 rpm and 37°C for 24 h. The synthesis of QDs were followed by visual observation of orange-yellow color formed in the culture, each pH was carried out in duplicate; no inoculum was added to sterile controls.

RESULTS AND DISCUSSION

The strain has a discriminatory ability to resist many metals such as selenium, cadmium, silver, zinc, copper, lead, nickel, cobalt and bismuth. It was observed that upon addition of the SeO_2 and $CdCl_2$ into the flask containing the TSB medium inoculated with *Providencia vermicola* BGRW, the color of the medium changed to yellow-orange color (Figure 1) and CdSe QDs were found extra/intracellularly; these QDs persisted for eight months in liquid suspension. BGRW strain was found to be highly resistant to selenium dioxide up to 20 mM and was able to grow in the presence of 6 mM of $CdCl_2$.

When cells containing the quantum dots at intracellular production, bacterial cells were collected, washed and sonicated or another easy procedure of nanoparticles bio-recovery was used, where the culture was sterilized at the ordinary autoclaving program. The wet heat sterilization process disrupted the bacterial cells containing the QDs; this method was used by Fesharaki et al. (2010) as a succeeding method for nanoparticles biorecovery by *Klebsiella pneumoniae* culture. Fluorescence spectrophotometer was used to compare the released QDs and the previous two methods (ultrasonication and autoclaving); the result confirms that autoclaving method had the higher released QDs from bacterial cells than ultrasonication method (Figure 2). The precipitate of nanoparticles was washed with ethanol and water many times to remove any contaminants present and dried in hot air oven at 45 -50°C; this step called purification from the protein and other contaminants formed in the reaction suspension (Shivashankarappa and Sanjay, 2015).

Characterization of CdSe QDs

Visualization of biogenic CdSe QDs color

For CdSe QDs biosynthesis by BGRW, the incubation of bacterial culture with sodium dioxide and cadmium chloride at 37° C for 24 h in dark showed a color change to bright orange-yellow (Figure 1, tube 2), which is the characteristic of CdSe QDs formation. This orange-yellow color is due to the excitation of surface Plasmon vibrations in CdSe QDs and it provides a convenient spectroscopic signature for CdSe QDs formation, whereas no color change could be demonstrated in a solution of selenium dioxide and cadmium chloride as a negative control (Figure 1, tube.1). Many related searches revealed that nanoparticles formation might involve a complex of either reductases, capping proteins, quinones or cytochromes,

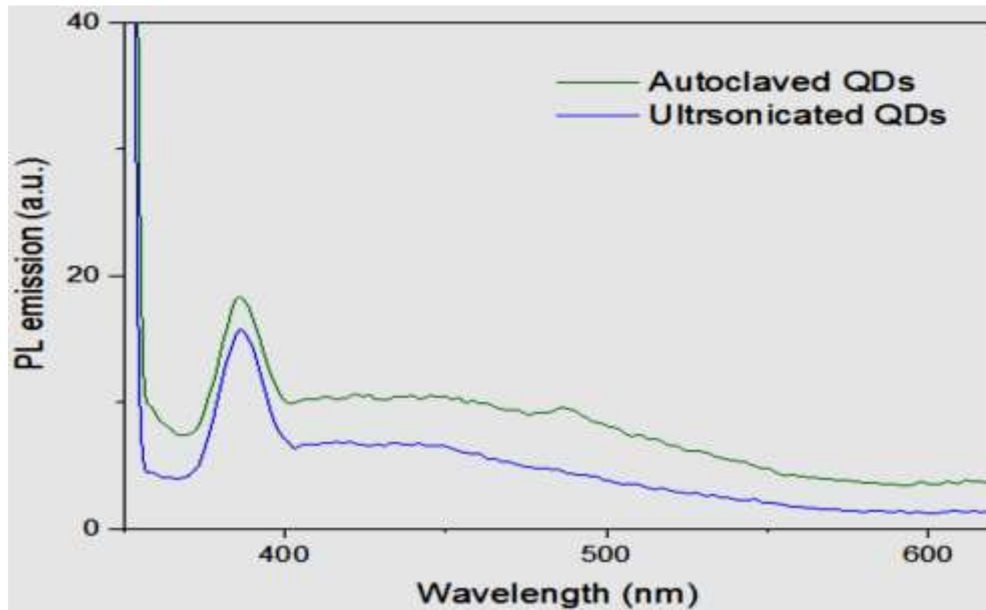

Figure 2. Fluorescence emission spectra of CdSe QDs suspension under excitation at 380 nm after biorecovery from bacterial cells by ultrasonication method and autoclaving method, an emission band centered at 385 nm.

Figure 3. A photograph of test tubes of the culture of *Providencia vermicola* BGRW with different concentrations of SeO_2 and $CdCl_2$ to CdSe QDs production.

electron shuttles or phytochelatins that are reduced and stabilized various metal nanoparticles (Kumar et al., 2007; Ayano et al., 2014). Furthermore, different size, shape and color of CdSe QDs formed depends on the molar ratio of cadmium and selenium ions in the medium; the culture with SeO_2 and $CdCl_2$ started to turn reddish brown color in presence of 0.5mM SeO_2:0.5 mM $CdCl_2$ within 24 h of incubation while in (0.1mM SeO_2:0.9mM $CdCl_2$) concentration, the color of culture was orange-yellow. Therefore, the CdSe QDs tends more yellowish when cadmium ions were increased in bacterial culture (Figure 3, tubes 3-5) while, the CdSe QDs tends more reddish when selenium ions were increased in bacterial culture (Figure 3, tubes 9-11).Colonies of BGRW strain formed orange-yellow colonies on the agar supplemented with (0.1 mM SeO_2:0.9 mM $CdCl_2$) after 24 h of incubation as shown in (Figure 4D). These results are in agreement with results obtained by other authors who concluded that different colors CdSe QDs are dependent on the molar ratio of Cd^{+2} and Se^{+2} in the medium. CdSe QDs

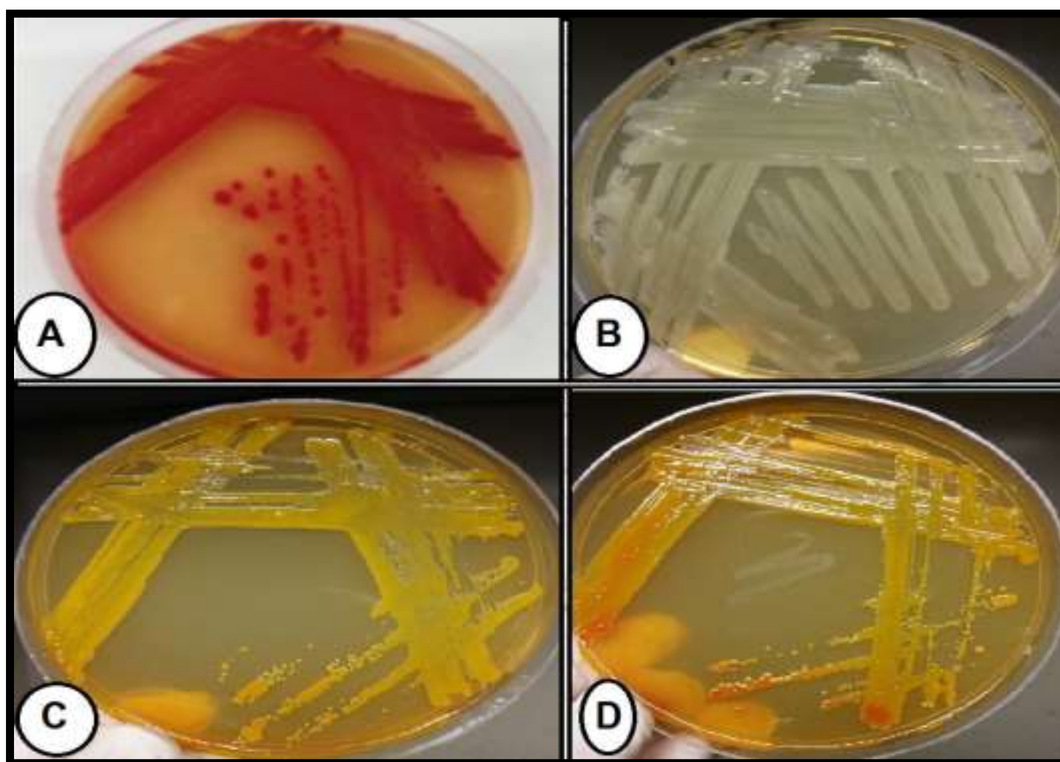

Figure 4. A digital photograph of *Providencia vermicola* BGRW on tryptic soy agar plates. **(A)** The red colonies indicate the production of selenium nanoparticles; **(B)** colonies without nanoparticles; **(C)** yellow colonies with cadmium nanoparticles; **(D)** orange- yellow colonies with CdSe QDs.

appeared reddish brown at (Kumar et al., 2007; Ayano et al., 2014) study, whereas the CdSe QDs appeared with light yellow color at (Suresh, 2014) study at 1 mM $CdCl_2$ and 1 mM $SeCl_4$ concentrations while SeNPs and CdS show dense red (Bajaj et al., 2012) and bright yellow (Ahmad et al., 2002), respectively.

Generally, the incubation period needed for the synthesis of CdSe QDs in the present study was 24 h when the others conditions were optimized: pH 9 and 37°C. In contrast, an incubation period required for the synthesis of CdSe QDs was 48 h by (Ayano et al., 2015).

In addition, an important observation that the reduction of the selenium and cadmium ions takes place extracellularly by *P. vermicola* BGRW as well as intracellular production was made. Accordingly, this offers a great advantage over an intracellular process of synthesis from the application point of view, since the CdSe QDs formed inside the bacteria would be needed for additional step of processing to ensure nanoparticles release from the bacteria using ultrasound treatment or by reaction with suitable detergents.

UV-Vis spectrophotometer for CdSe QDs

For CdSe QDs spectra measurements, the UV-Vis spectrum illustrated shows an absorption peak in the region of 200-800 nm for the cell filtrate from the culture of *P. vermicola* BGRW strain which had 0.1 mM SeO_2: 0.9 mM $CdCl_2$ as a source of selenium and cadmium ions. This showed the presence of absorbance bands centered at 388 nm, suggesting the formation of CdSe quantum dots, which is (Figure 5) compatible with the study of Ogermann et al. (2012). Similar to spectra of CdSe, QDs showed the presence of absorbance band centered at 350 nm in Gupta and Ramrakhiani (2009) and Suresh (2014) studies. When the biological method of CdSe QDs production was used, an absorption band may be centered between 270 and 280 nm, which is visible due to protein or peptide, associated with the quantum dots; indicating a possible protein or a peptide core-shell as a capping molecule (Wu et al., 2015). The broad peak of QDs may be due to the variation in the size of the nanoparticles (Malarkodi et al., 2014). However, some of the other studies did not use UV-Vis spectrophotometer to characterize CdSe QDs (Cui et al., 2009; Ayano et al., 2015).

Fluorescence spectrophotometer for CdSe QDs

Depending on fluorescence property of quantum dots, photoluminescence characteristics of the biogenic CdSe quantum dots were studied by fluorescence spectral

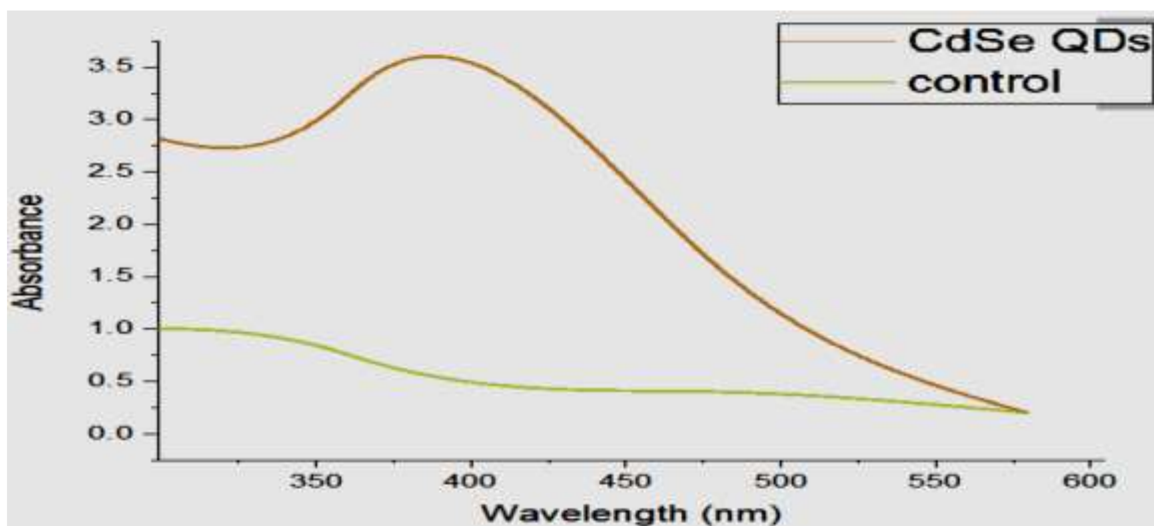

Figure 5. UV-Vis spectra recorded of CdSe QDs produced by *Providencia vermicola* BGRW, The absorption spectrum of CdSe QDs exhibited strong peaks at 388 nm and UV-spectra of cell-free supernatant was also represented as a control.

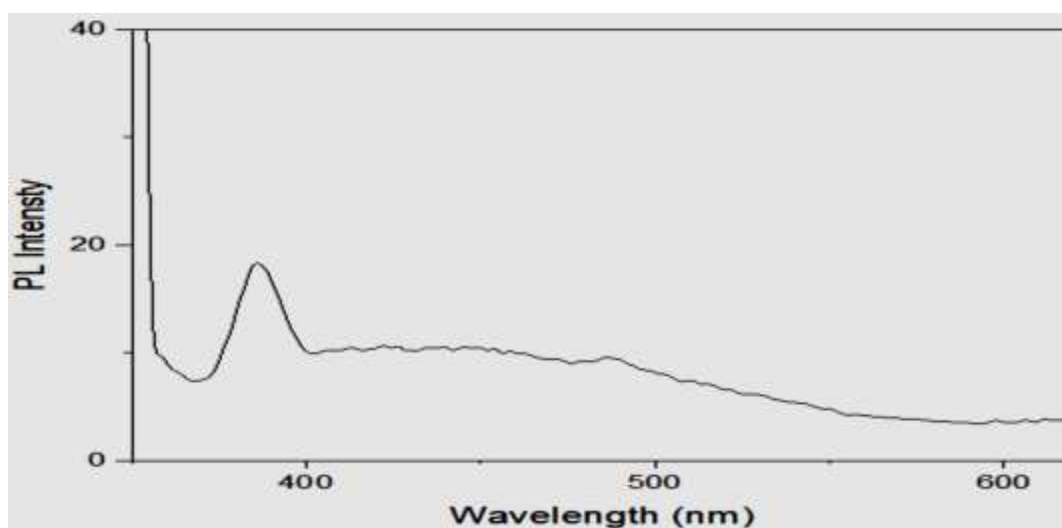

Figure 6. Fluorescence emission spectra of CdSe QDs produced by *Providencia vermicola* BGRW, a suspension under excitation at 380 nm, and an emission band centered at 385 nm.

measurements upon excitation at 380 nm. An emission band centered at 385 nm (Figure 6) was observed and was comparable to the emission peaks of QDs synthesized by chemical method reported earlier (Zhang et al., 2007; Fokina et al., 2016).

The size-dependent absorbance and fluorescence spectrum in the visible range, commonly observed for similar quantum dots was not detected at (Cui et al., 2009; Qu et al., 2001) studies. Ayano et al. (2014) explained why different wavelengths absorbed and emitted, whereas a strong absorbance (300–400 nm) and fluorescence (390–560 nm) was observed, there was probability that the CdSe quantum dots spectrum might have been overlapped with one or varied bio-molecules such as $NADH/NAD^+$, whose absorbance and fluorescence might have been correlated with CdSeQDs.

The emission is referred to the band gap or near band gap emission, resulting from the recombination of electron-hole pairs in the quantum dot nanoparticles (Smith and Nie, 2009). The absorption spectrum (Figure 7) of CdSe QDs did not show bands at wavelengths longer than 400 nm. Upon ultraviolet irradiation, however, an emission centered at 385 nm appears in the spectrum in Figure 7.

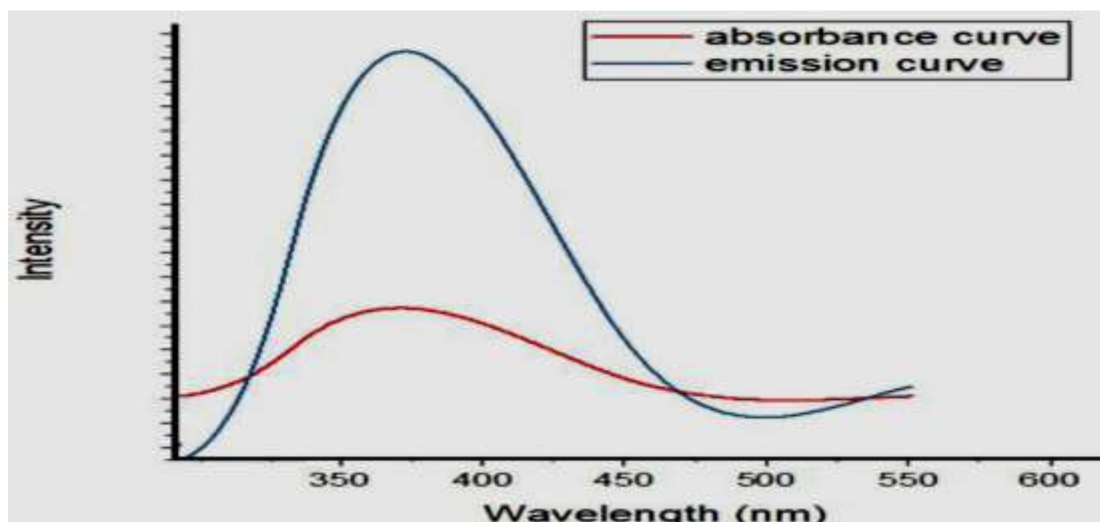

Figure 7. UV-Vis and fluorescence spectra recorded of CdSe QDs produced by *Providencia vermicola* BGRW, the absorption spectrum of CdSe QDs exhibited strong peaks at 388 nm and emission spectrum peak under 380 nm excitation appeared about 385 nm.

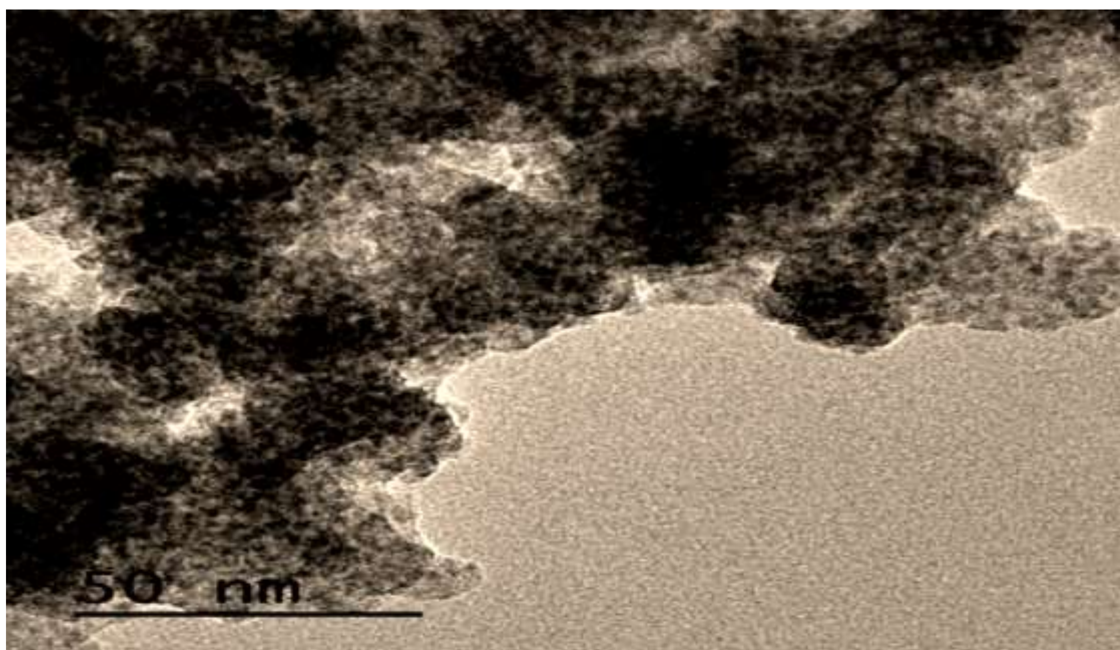

Figure 8. TEM image of the CdSe QDs produced by the reaction of 0.1 mM SeO_2:0.9 mM $CdCl_2$ ratio with bacteria *Providencia vermicola* BGRW culture incubate for 24 h.

Transmission Electron Microscopy (TEM) measurements of CdSe QDs

TEM images of the CdSe QDs synthesized by a culture of *Providencia vermicola* BGRW with 0.1 mM SeO_2:0.9 mM $CdCl_2$ ratio were done in Figure 8, the particles ranged in size approximately from 2 to 4 nm in diameter in one vessel production. Also, transmission electron micrographs of prepared CdSe QDs (Figure 8) showed that the particles were poly-dispersed nanoparticles. Cui et al. (2009) reported the smallest size of CdSe QDs produced by *Saccharomyces cerevisiae* yeast, pluralities of the particles were monodispersed spheres with a mean diameter of 2.69±0.07 nm, while in Ayano et al. (2014) study, the CdSe QDs produced by *Pseudomonas* sp. strain RB were 10-20 nm. Recently, 15 to 20 nm CdSe

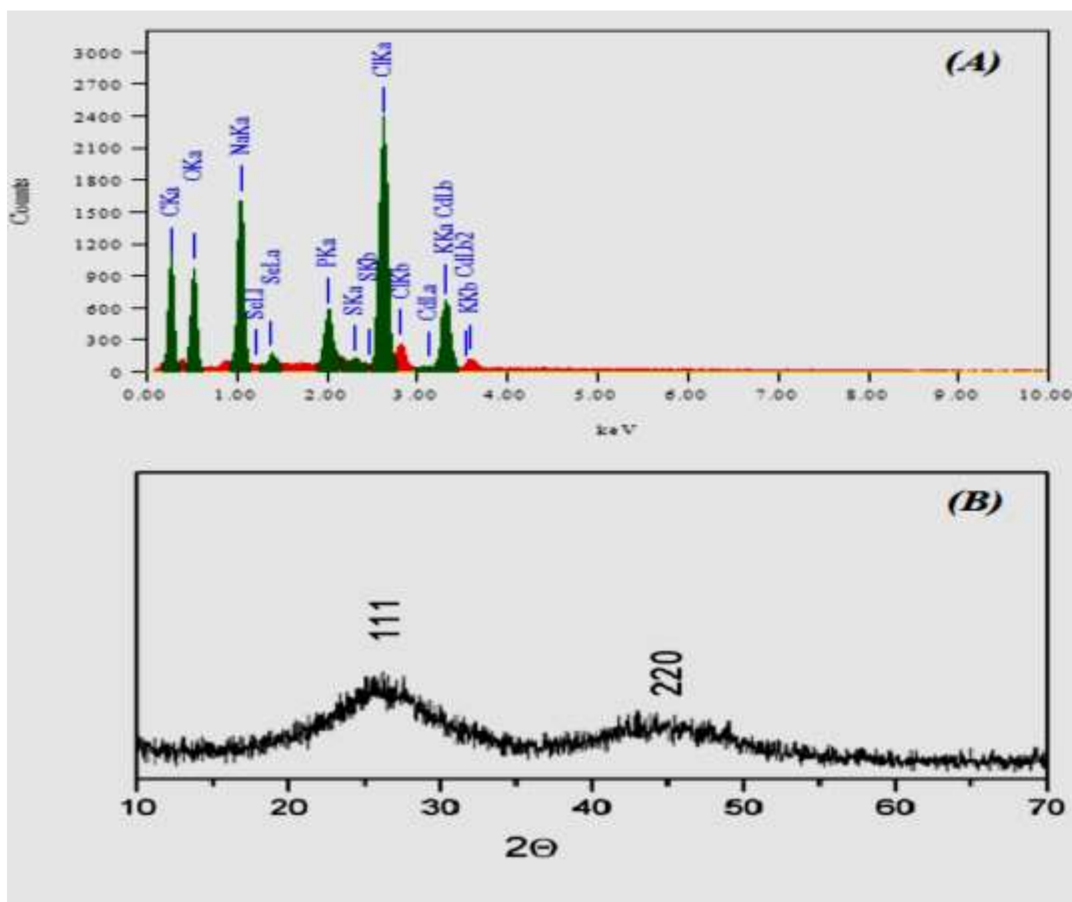

Figure 9. Representative EDS pattern (A) and XRD spectra (B) of CdSe QDs synthesized by the reaction of *Providencia vermicola* BGRW culture with 0.1 mM SeO_2: 0.9 $CdCl_2$ ratio.

QDs were biosynthesized by yeast (Wu et al., 2015).

Energy-dispersive X-ray (EDS) analysis and X-Ray diffraction (XRD) analysis of CdSe QDs

Furthermore, energy-dispersive x-ray (EDS) analysis of freeze-dried CdSe QDs is shown in Figure 9A. The EDS result of the CdSe QDs confirmed that the QDs contained elements such as cadmium, selenium, oxygen, carbon, sulphur, sodium, phosphorus, chlor and potassium. The existing elements oxygen, carbon, phosphorus and sulphur, which are the main integral of proteins and oligopeptides took a step closer to this hypothesis, CdSe QDs were manufactured with the assistance of protein molecules and on the protein layer, giving the CdSe products high stability (Srivastava and Mukhopadhyay, 2013).

In addition, the XRD pattern indicates CdSe quantum dots exhibit cubic crystal structure. The intensity of XRD peaks was not strong and little broad indicates the semi-amorphous nature of the biosynthesized CdSe QDs (Figure 9B).

Fourier transforms infrared spectroscopy (FTIR) analysis of CdSe QDs

Biosynthesized CdSe QDs that were produced by *Providencia vermicola* BGRW was characterized by using attenuated total reflection Fourier transform infrared (ATR-FTIR). The ATR-FTIR spectrum (Figure 10) of bacterial crude protein and the CdSe QDs surface showed obvious changes in both the shape and the peak position, suggesting the changes in the secondary structure of protein after nanoparticle formation. FTIR results revealed that secondary structures of proteins were affected as a consequence of binding with CdSe QDs. ATR- FTIR spectra of CdSe QDs (Figure 10) showed that several peaks appeared at 650, 695, 905, 1063, 1195, 1234, 1328, 1540, 1634, 2230, 2324, 2391, 2646, 2924, 3068, 3272, 3662 and 3774 , which are characteristic of proteins. The strong broad peaks at 2646, 2924, 3068, 3272, 3662 and 3774 can be corresponding to the amine group (NH stretching) and 3272, 3662 and 3774 cm^{-1} can be assigned to hydroxyl (OH) group. The peaks at 695, 2924, 3068 and 1540 are associated with C-H stretching (Mukherje, 2014). The bands at 1063, 1195 and 1234 may

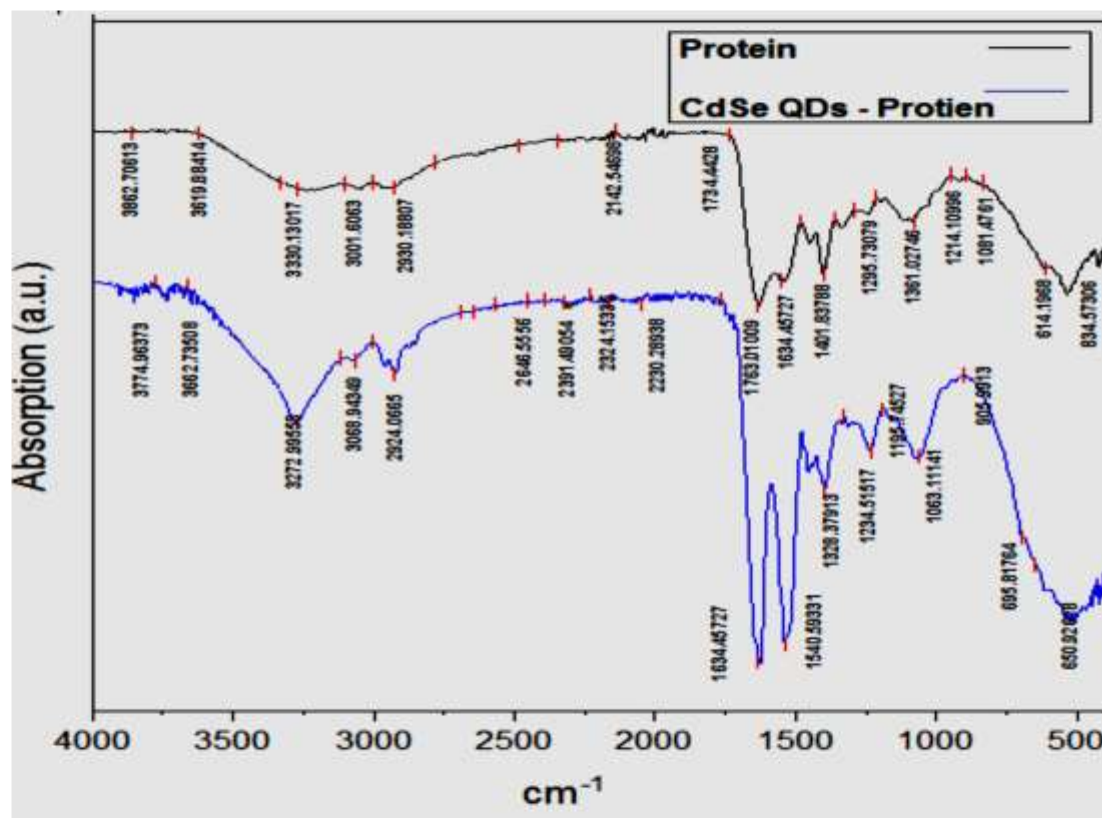

Figure 10. A representative ATR-FTIR spectrum pattern of dried powder of CdSe QDs synthesized by the reaction of 0.1 mM SeO_2:0.9 mM $CdCl_2$ ratio with bacteria *Providencia vermicola* BGRW culture at pH 7.0.

be attributed to C-O stretching mode (Huang et al., 2007). The peak at 695 likely due to the presence of aromatic compounds (Tani et al., 2007). Furthermore, the FTIR spectrum revealed two bands at 1634 and 1540 cm^{-1} corresponding to the amide I and II bands of proteins, respectively. The Amide I band is primarily a C=N stretching mode and the Amide II band is a combination of N-H in-plane bending and C-N stretching. The more-complex Amide III band is located near 1328 cm^{-1}. The amide groups indicating the presence of proteins as mentioned above. With the overall observations, it can be concluded that the proteins might have formed a capping agent over the CdSe QDs, which may respond to their stabilization (Dhar et al., 2015). Therefore, the produced CdSe QDs persisted for several months in liquid suspension. The peak at 650 cm^{-1} corresponds to C−S disulfide stretching vibration indicate the frequent occurrence of thiols and its substituted compounds, constituting the backbone of the interacting protein. From the ATR-FTIR spectra, an interaction between CdSe QDs and protein is farther confirmed by the shift in CS (614 cm^{-1} to 1328 cm^{-1}), CO (1081 cm^{-1} to 1063 cm^{-1}), CN (1295 cm^{-1} to 1234 cm^{-1}), nitro compound (1401 cm^{-1} to 618 cm^{-1}) and OH (2930, 3001, 3330, 3619 and 3862 cm^{-1} to 2924, 3068, 3272, 3662 and 3774 cm^{-1}).

Some of these peaks (1540, 3662 and 3774) revered hydroxyl, amide and amine which were similar between selenium nanoparticles and CdSe QDs. This means that the capping agent for different nanoparticles were similar if the bacterial strain was one, while there were a lot of various peaks at QDs. It is worth mentioning that, there are no previous studies characterizing the capping agent of biosynthesized CdSe QDs. At similar study on CdS QDs, the stability of the CdS QDs is brought by the binding of the proteins to nanoparticles, either by free amino groups or through cysteine residues (Sanghi and Verma, 2009; Ahmad et al., 2002). The data provide some insights on the nature of the capping layer of nanoparticles surface, which may be further utilized for bio-functionalization for different applications (Syed and Ahmad, 2013).

Optimization the condition of CdSe QDs synthesis

The effect of temperature on CdSe QDs formation

Several factors can affect the stability, size, shape, crystallinity and aggregation of QDs, among these factors are incubation time of bacteria with precursors, precursor concentration, biomass, type of bacteria, the presence of oxygen, presence of capping agent, a negative charge of

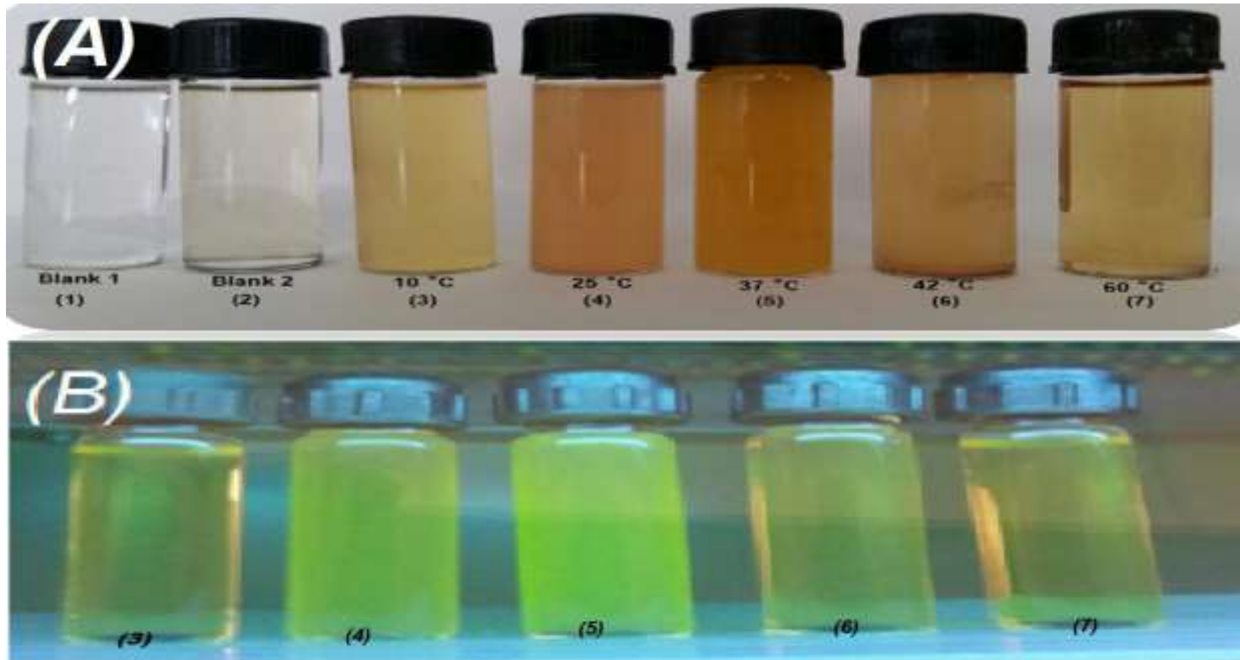

Figure 11. (A) Photograph of different colors obtained through the CdSe QDs production at varying temperature values. The tubes' numbers (3-7) indicate *Providencia vermicola* BGRW culture reactions with 0.1 mM SeO_2:0.9 $CdCl_2$ and pH 7.0 incubation at 10, 25, 37, 42 and 60°C, the numbers (1-2) the corresponding blank reactions; **(B)** photograph shows fluorescence emission of CdSe QDs reactions (3-7) under UV lamp, indicating CdSe QDs optimum production at 37°C.

NPs, pH, temperature and presence of EPS and PVP (Tam et al., 2010). One of the important parameters to evaluate the efficiency of the biosynthesis method is the incubation temperature, the rate of any chemical and biological reaction is affected by it. Quantum dots synthesis of *Providencia vermicola* BGRW culture in terms of color intensity were examined at different temperature (10-60°C). The increase in the color intensity of culture was due to increased number of quantum dots formed (Shirsat et al., 2016). In addition, the increase in fluorescence intensity under UV-lamp of culture was due to increasing number of CdSe QDs (Ayano et al., 2014). In addition, green synthesis of CdSe QDs is enzyme-mediated process and enzymes are temperature sensitive.

The results were found for CdSe QDs formation started at 25°C (Tube 3), with the optimum formation at 37°C (Tube 5) (Figure 11A). It was found that changes in temperature greatly affected the rate of QDs biosynthesis at 0.9 mM $CdCl_2$: 0.1 mM SeO_2 ratio and pH 7. At 10°C, the culture reaction did not give a color of CdSe QDs, signifying no formation of CdSe QDs; indicating that chemical reduction was not formed. At 25 to 37°C (Tubes 4 and 5), the culture gave an intense orange-yellow color, with a clear difference in the blank reaction, proving it to be the optimum temperature to produce QDs. At 42 and 60°C (Tube 6) the culture did not give color, again a clear difference in the blank reaction, these results were also confirmed under UV-lamp (Figure 11B); whereas the culture incubated at 37°C showed the highest intensity of fluorescence emission. Also, UV-Vis spectroscopy affirmed these results as shown (Figure 12), showed that the optimum temperature for the synthesis of QDs by *Providencia vermicola* BGRW was 37°C, whereas the control experiment, that is SeO_2 and $CdCl_2$ solution incubated at different temperature (10-60°C) showed no sign of QDs synthesis (data not shown). A similar study, Ayano et al. (2014), reported that the optimum temperature for growth that is most practical is expected to be 37°C because the specific growth rate declined at temperatures higher than 40°C. Also, the eliminations of selenite and cadmium ion become slower as the temperature rose, *Pseudomonas aeruginosa* Strain RB grew well with removal of most of the supplemented amounts of selenite and cadmium at 30°C, although growth happened more rapidly at 37°C than that at 30°C.

The effect of metal ion concentration on CdSe QDs formation

The effect of different SeO_2 and $CdCl_2$ ratios (concentrations of SeO_2 and $CdCl_2$) (0.1 mM:0.9 mM, 0.2 mM:0.8 mM, 0.3 mM:0.7 mM, 0.4 mM:0.6 mM, 0.5 mM:0.5 mM, 0.6 mM:0.4 mM, 0.7 mM:0.3 mM, 0.8 mM:0.2 mM and 0.9 mM:0.1 mM) were investigated. Flasks containing the reaction mixtures of bacterial culture incubated with different ratios of SeO_2 and $CdCl_2$ compared to four control. The first one was the bacterial culture with SeO_2, the second was the aqueous solution of SeO_2, the third

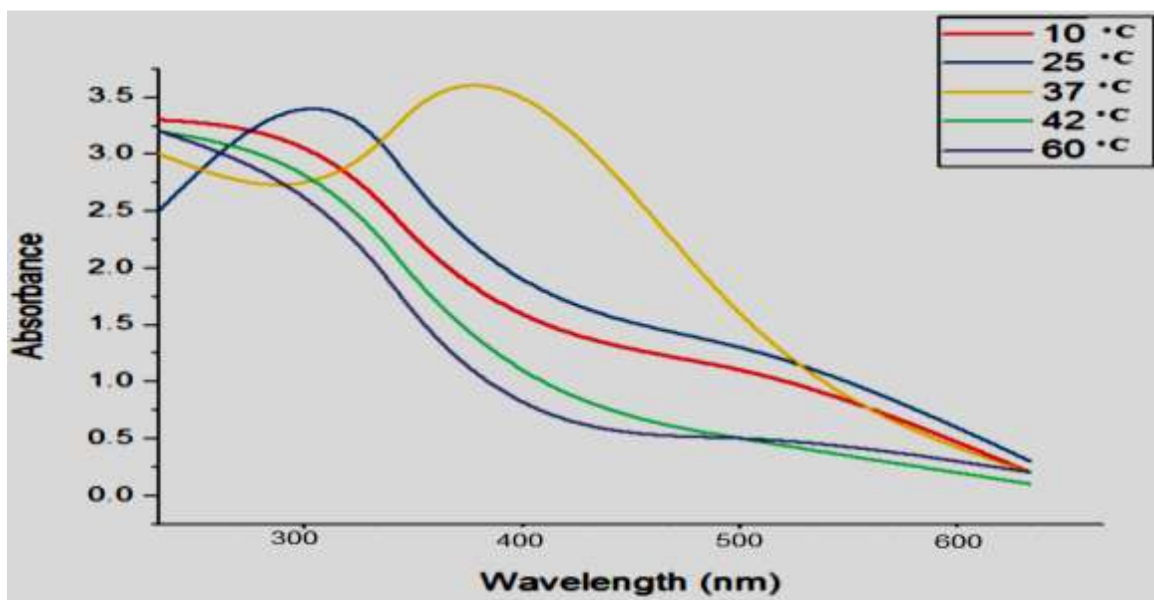

Figure 12. UV-Vis absorption spectra of the surface Plasmon resonance recorded for the different temperature values, indicating CdSe QDs production through a change in the absorbance around 388 nm.

was the bacterial culture with $CdCl_2$, and the last was the aqueous solution of $CdCl_2$. Visual analysis revealed that the orange-yellow color changed with different ratios of selenium and cadmium ions concentrations (Figure 13A); whereas, the CdSe QDs tends to be more yellowish when cadmium ions were increased in bacterial culture (Tubes 3-5) from (Figure 13A). However, the CdSe QDs tends to be more reddish when selenium ions were increased in bacterial culture (Tubes 8-11) from (Figure 13, A). When these tubes were exposed to the UV light (Figure 13B), fluorescent properties which distinguished quantum dots clearly appeared in the following concentrations of $CdCl_2$ and SeO_2 (0.4 mM:0.6 mM, 0.5 mM:0.5 mM, 0.6 mM:0.4 mM, 0.7 mM:0.3 mM, 0.8 mM:0.2 mM and 0.9 mM:0.1 mM), respectively.

The optimum ratio of CdSe QDs production, which had the highest fluorescence emission, was 0.9 mM $CdCl_2$:0.1 mM SeO_2 investigated by quickly measuring the maximum fluorescence intensities of QDs contained bacteria solutions to determine the optimal synthetic conditions. The emitted fluorescence light increase with high concentration of cadmium ions and low concentration of selenium ions. It is worth mentioning that, the reaction of *P. vermicola* BGRW culture with cadmium chloride (Tube 2) at (Figure 13B) did not emit any fluorescence light under UV light which shows the quantum dots did not produce, unless the existence of cadmium and selenium ions together and the type of QDs were CdSe, not CdS. In addition, it is worth mentioning that the color of fluorescent lighting is different by various ratios, which showed that the concentration of materials effect on the size of the quantum as known, the fluorescent color differs depending on the QDs size similar to other results (Wu et al., 2015). Whereas, the CdSe QDs tends to give green fluorescent when cadmium ions were increased in bacterial culture (Tubes 3-5), (Figure 13B), the CdSe QDs tends to orange fluorescent when selenium ions were increased (Tubes 9, 10) (Figure 13B).

These results were also confirmed by fluorescence spectroscopy, Figure 14 shows the spectra obtained when bacterial culture was incubated with varying ratios of SeO_2 and $CdCl_2$. As shown in (Figurd 14), the fluorescence spectra recorded from the bacterial culture to determine CdSe QDs, in all ratios of SeO_2 and $CdCl_2$ showed the appearance strong emission peak centered at about 385 nm, which is a characteristic for CdSe QDs. Furthermore, the increasing intensity of the peak can be attributed to the increase in the number of QDs in the solution. In this study, the CdSe QDs production by BGRW increased according to cadmium ions increased. The fluorescence spectra was used to determine the optimized ratio of CdSe QDs production instead of UV-Vis spectra because the estimation of the highest absorption of ratios used was difficult; referring to the presence of many peaks at many positions.

In another study, Yan et al. (2014) produced CdSe QDs from *E. coli* cells which had the strongest ability to biosynthesize QDs when 250 μM selenium and 750 μM cadmium concentration was used. Yan et al. (2014) resulted that, high concentrations of Na_2SeO_3 or $CdCl_2$ might contribute to superoxide anion oxidative damage, which would reduce the intracellular biochemical activity, leading to the decrement of QDs biosynthesis. In addition, when the concentration of selenium or cadmium was at a low level, the synthesis ability of cells was not saturated, leading to a low yield of CdSe QDs.

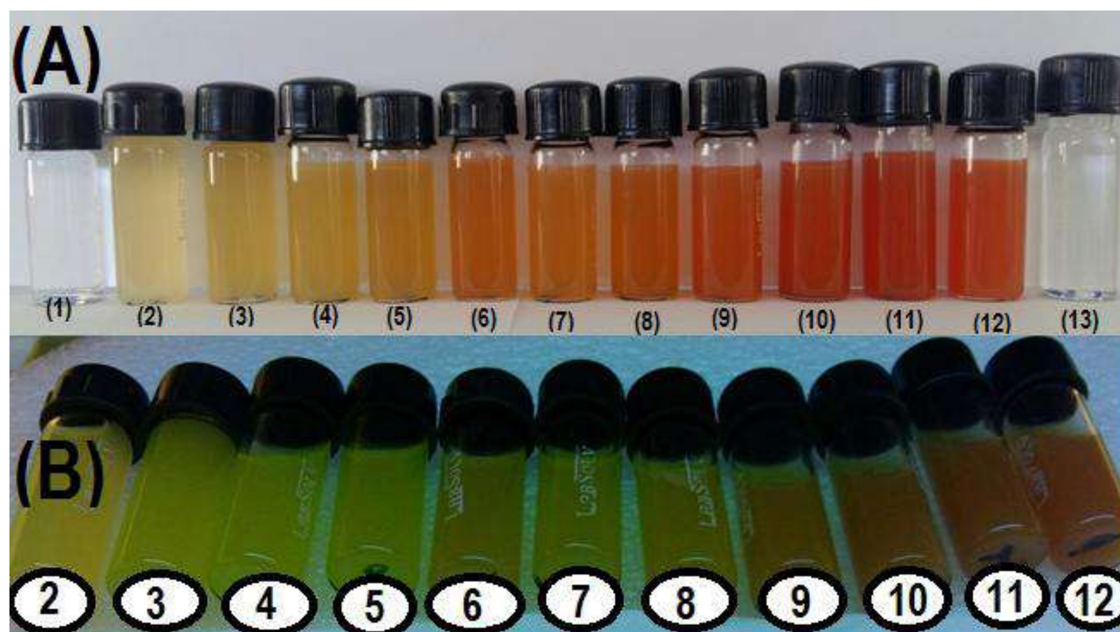

Figure 13. **(A)** Digital photograph of test tubes culture of *Providencia vermicola* BGRW with different concentrations of SeO_2 and $CdCl_2$ to CdSe QDs production: (tube 1) aqueous solution of $CdCl_2$, (tube 2) culture of *Providencia vermicola* BGRW with 1 mM $CdCl_2$, (tube 3) culture with 0.1 mM SeO_2:0.9 mM $CdCl_2$, (tube 4) culture with 0.2 mM SeO_2:0.8 mM $CdCl_2$, (tube 5) culture with 0.3 mM SeO_2:0.7 mM $CdCl_2$, (tube 6) culture with 0.4 mM SeO_2:0.6 mM $CdCl_2$, (tube 7) culture with 0.5 mM SeO_2:0.5 mM $CdCl_2$, (tube 8) culture with 0.6 mM SeO_2:0.4 mM $CdCl_2$, (tube 9) culture with 0.7 mM SeO_2:0.3 mM $CdCl_2$, (tube 10) culture with 0.8 mM SeO_2:0.2 mM $CdCl_2$, (tube 11) culture with 0.9 mM SeO_2:0.1 mM $CdCl_2$, (tube 12) culture with 1 mM SeO_2, (tube 13) aqueous solution of SeO_2. All test tubes were incubated for 24 h at 37°C; **(B)** digital photograph of (2-12) previous test tubes under UV light showed the fluorescence property of biosynthesis QDs.

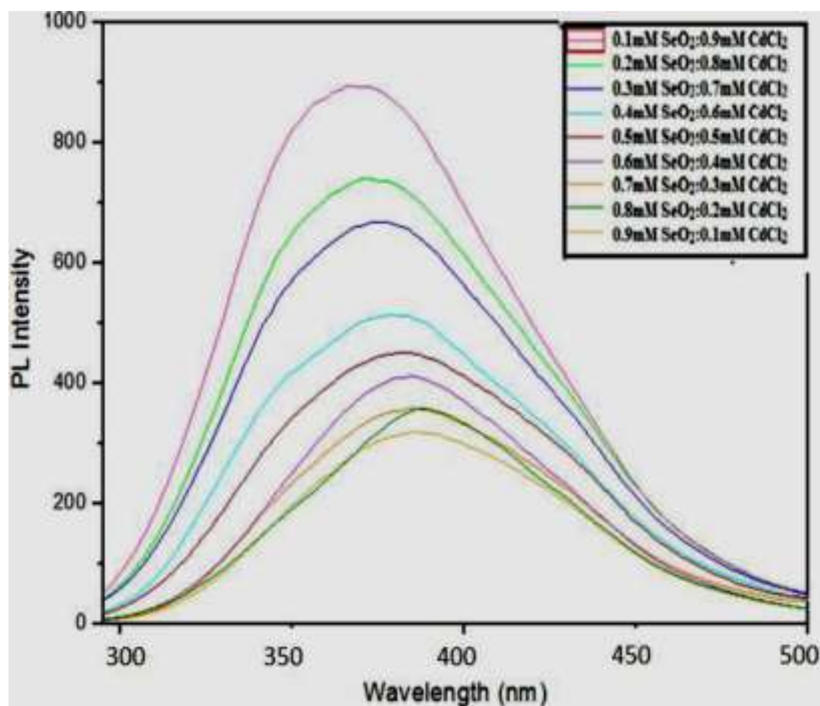

Figure 14. Fluorescence emission spectrum of CdSe QDs solution under excitation at 380 nm, after the reaction of different ratios of SeO_2 and $CdCl_2$ solution with the bacteria *Providencia vermicola* BGRW culture for 24 h at neutral pH, single and strong emission peak centered at about 385 nm.

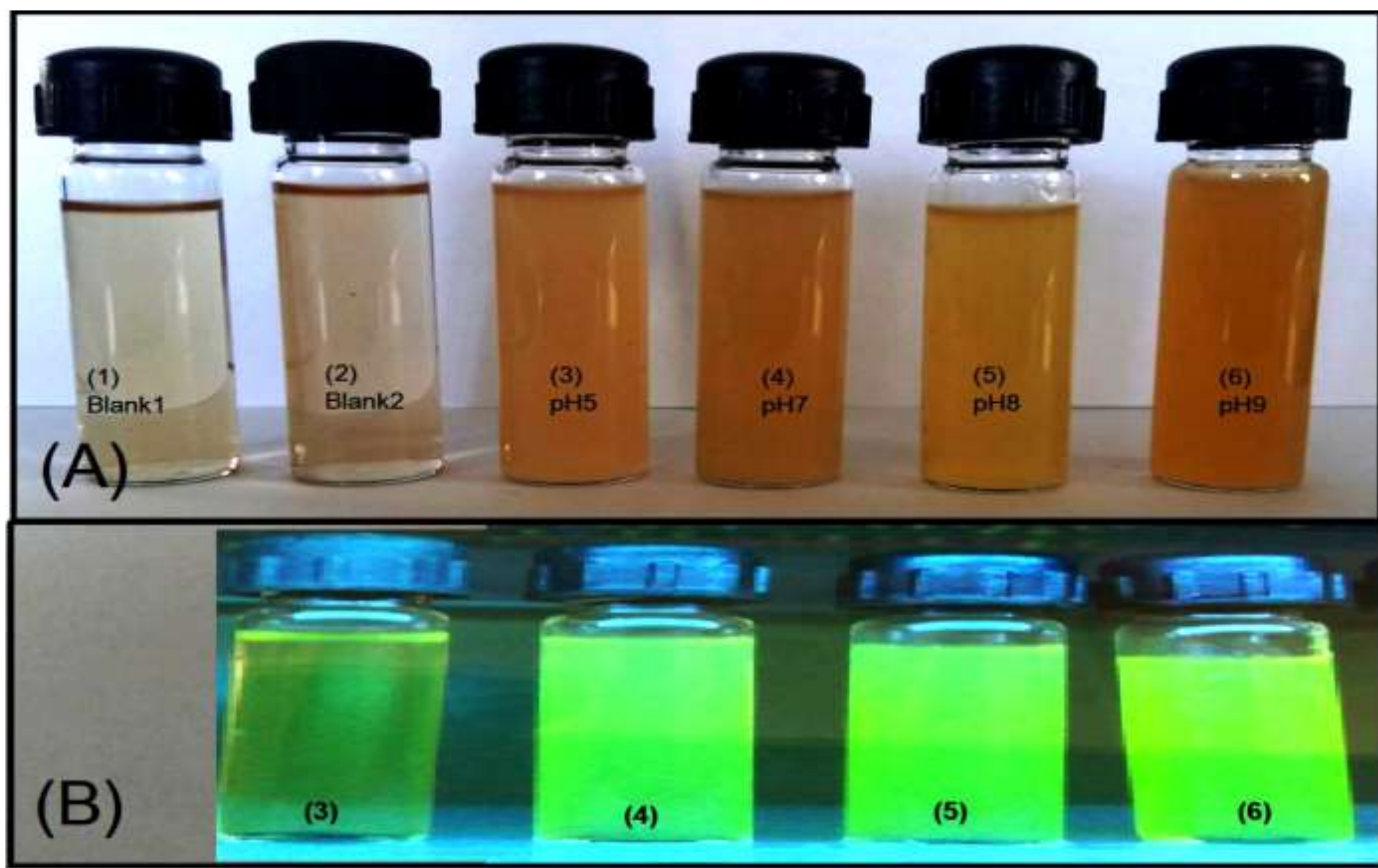

Figure 15. (A) A photograph showing different colors obtained through the CdSe QDs production at varying pH values, the tubes' numbers (3-6) indicate *Providencia vermicola* BGRW culture reactions with 0.1 mM SeO_2:0.9 $CdCl_2$ and tubes (1 and 2) correspond to blank reactions; (B) A photograph showing fluorescence emission of CdSe QDs solution under UV-lamp at different pHs.

The effect of pH on CdSe QDs formation

Furthermore, CdSe QDs were formed at various pH values (5.0, 7.0, 8.0 and 9.0), the optimum formation of QDs was at pH 9.0 (Tube 5) of (Figure 15). When pH was increased from 5 to 9, maximum synthesis was observed at pH 9.0. The synthesis time was greatly reduced and the yield of QDs was significantly enhanced, as evidenced by UV-Vis spectroscopy (Figure 16). The color of culture at pH 5 was more reddish than that of the other cultures under neutral and alkaline pHs. The bacterial culture at pH 8.0 actually became yellower than those under neutral or acidic conditions are which is similar to a previous report (Ayano et al., 2015).

Therefore, the present study shows that the optimum pH for the synthesis of QDs was 9.0. The control experiments, that is, a mixture of SeO_2 and $CdCl_2$ solution incubated at different pH (5-9) showed no synthesis of QDs (data not shown).

This is also in agreement with earlier reports that addition of an alkaline ion is necessary to carry out the reduction reaction of metal ions (Shankar et al., 2004) and in the absence of the hydroxyl ion, the time required for reduction of metal ions was prolonged.

There was one report that discussed the effect of pH condition on CdSe QDs production by bacteria (Ayano et al., 2014). Their results indicated that the selenite and cadmium removals did not decrease until 24 h at pH 6.5 and were slower thereafter, that was not applicable to the CdSe synthesis. At pH 8.0, the removal of both selenite and cadmium decreased considerably and the cadmium removal was higher than the selenite removal, this tendency was also observed at pH 8.5.

Conclusion

The microbial mediated synthesis of CdSe QDs may replace some of the current physical and chemical methods for metal nanoparticles production. *P. vermicola* BGRW has the potential to be used as a bio-Nano factory for the synthesis of stable cubic CdSe QDs, ranging from ~2 to 4 nm. Optimization of CdSe QDs synthesis was studied and the results demonstrated that the best ratio for CdSe QDs production was 0.1mM SeO2:0.9mM CdCl2 and the paramount other conditions were pH 9 and 37°C.

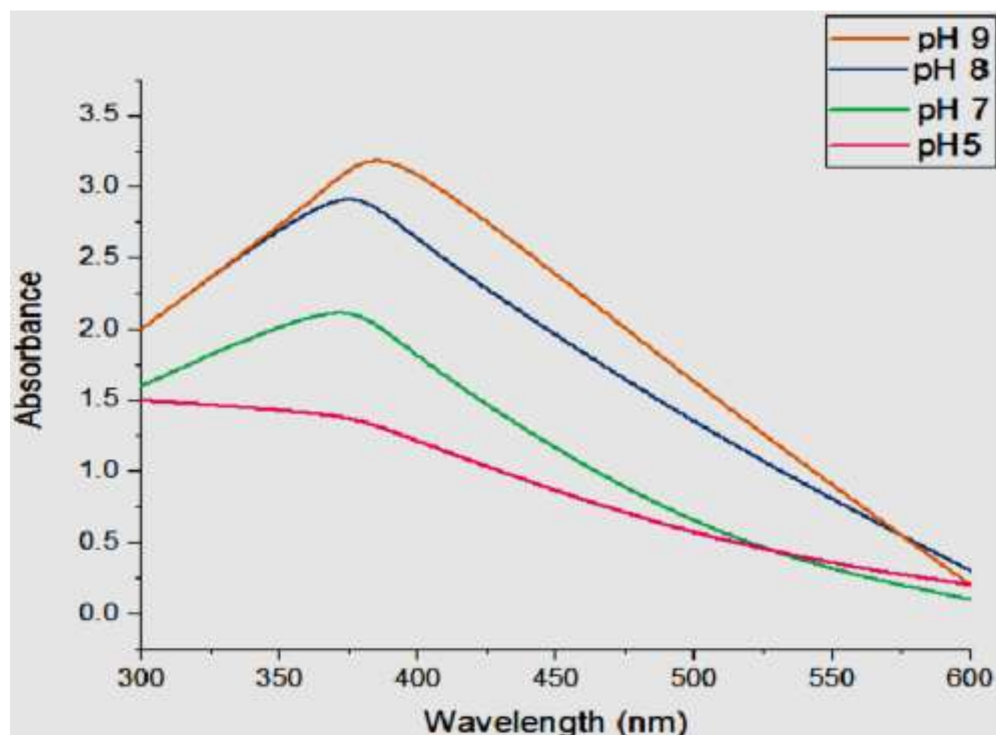

Figure 16. UV-Vis absorption spectra of the surface Plasmon resonance recorded for the different pH values, indicating CdSe QDs production through a change in the absorbance around 388 nm.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Influence of aqueous extracts of black angico on *Pratylenchus brachyurus* in cotton plants

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The root lesion nematode (*Pratylenchus brachyurus*) is one of the main phytosanitary problems of cotton plants in Brazil. Searching for alternatives that minimize the damages in the crop, several methods are performed aiming to manage these damages. Among them, is the use of vegetal extracts. In this sense, the aim of this study was to evaluate the potential of black angico extract (*Anadenanthera macrocarpa*) in the management of *P. brachyurus* in cotton crop. The experiment was conducted in a greenhouse at the Phytopathology Laboratory of the Federal University of Piauí in Bom Jesus-PI. The experimental design was completely randomized, in a factorial scheme (2×6), composed of two sources of extracts (leaf and bark) of black angico under six concentrations (0, 20, 40, 60, 80 and 100 g L^{-1}), with five replications per treatment. The plants were inoculated with 1900 specimen/juveniles and eggs, 96 h after the transplanting. Sixty days after the application of extracts, some agronomic variables of the cotton and *P. brachyurus* were evaluated. The volume and fresh root mass showed considerable gains for all concentrations with the leaf extract. The plant height was negatively influenced by concentrations above 60.83 g L^{-1} for both extracts. Regarding the parasitism, all the extract concentrations, regardless of the source (leaf or bark), showed suppressiveness to *P. brachyurus*. Therefore, the aqueous extracts of black angico present nematicidal action and favor the development of cotton plants.

Key words: *Gossypium hirsutum*, root lesion nematode, alternative management.

INTRODUCTION

The cotton crop (*Gossypium hirsutum* L.) represents one of the most important activities of Brazilian agribusiness in a vigorous expansion process and with exceptional technical and economic results for the use of its seed and

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mainly its fiber (Ribeiro et al., 2012). In the 2015/2016 season, the cotton planted are decreased by 2% compared to the previous harvest, with an area of 956.2 thousand hectares (CONAB, 2016).

Cotton is grown in more than 60 countries. Anobg, China, India and the United States are the largest producers and together they produce 64% of the world production. Despite having larger planted area, India produces a volume of fibers almost equal to the United States due to the low yield of its crops. The list of the top five producers is completed by Pakistan and Brazil. In recent years, Brazil has improved its ranking in producing countries. Currently, it is the fifth largest producer in the world (Abrapa, 2015).

Among the barriers to cotton crop management, phytosanitary problems caused by fungi, bacteria, viruses and mainly nematodes are often associated with reduced low crop yield (Ribeiro et al., 2012). Among the key nematodes of this crop, there are approximately five species responsible for causing severe damage worldwide. Three of them are considered as causing significant damages to Brazilian cotton production: *Meloidogyne incognita, Rotylenchulus reniformis, Pratylenchus brachyurus* (Starr et al., 2007; Jones et al., 2013).

Currently, the nematode *P. brachyurus,* which is responsible for root lesions, has been considered the most frequent in Brazil and is widespread in the main agricultural regions of the country (Severino et al., 2010). Because it is a polyphagous species and is extremely common in regions of tropical climate (Arieira et al., 2009), and has become a concern to cotton producers in the Northeast region. It is the third most important regarding the global economic impacts caused to crops, being exceeded only by root-knot and cyst nematodes (*Heterodera* and *Globodera*) (Jones et al., 2013). The symptoms associated with this species in cotton include darkened injuries on the roots, causing atrophy, and may even compromise the absorption of water and nutrients (Dinardo-Miranda et al., 2003) and consequently, reduction in the shoot part development of plants with a sharp drop in production (Ribeiro et al., 2012).

Considering the great importance of phytonematodes management in commercial production areas, chemical control has always stood out because of its fast and efficient results (Oliveira et al., 2005). However, numerous problems are encountered due to their high toxicity, risk of environmental contamination, high cost, or low control effectiveness after repeated applications (Dong and Hang, 2006). In an attempt to reduce these effects, different control methods such as genetic, biological, crop rotation and alternative control have been studied.

Within this context, vegetable extracts represent a viable alternative to alleviate economic and social conditions of most of the farmer. In addition, their use reduces or replaces chemical application (Ferraz et al., 2010). Several studies have demonstrated the nematicidal effect of extracts of different plants on different species of phytonematodes when applied directly to the soil or by air (Cetintas and Yarba, 2010). Among these species, the black angico (*Anadenanthera macrocarpa*) is worth mentioning because it has potential to manage several diseases in the human, animal and plant area, with emphasis to phytonematodes. Black angico is a tree that can reach 13 to 20 m of height and trunk with 40 to 60 cm of diameter, when adult, occurring from Maranhão and Brazilian Northeast to São Paulo, Minas Gerais and Mato Grosso (Gonçalves et al., 2012).

Thus, the aim of this study was to evaluate the potential of plant extracts based on black angico (*A. macrocarpa*) on the management of *P. brachyurus* in cotton.

MATERIALS AND METHODS

Location of the experimental area and soil treatment

The experiment was performed under greenhouse conditions at the Phytopathology Laboratory, at the Universidade Federal do Piauí, Prof Cinobelina Elvas campus, Bom Jesus city, from October to December, 2014.

To evaluate the treatments, the substrate was composed of soil-sand-manure in a proportion of 3:2:1, respectively. It was autoclaved, at a temperature of 120°C and pressure of 1.05 kg/cm^2 for 2 h. The substrate was fertilized according to the previous analysis and distributed into plastic containers with capacity of 4 dm^{-3}.

Origin and multiplication of inoculum

The inoculum was obtained from a population of *P. brachyurus* from soybean crops in Bom Jesus-PI. The extraction was carried out by liquefaction and centrifugation in sucrose with kaolin solution, according to Coolen and D'Herde's (1972) methodology. Soon after, the specimens were isolated and inoculated in corn hybrid plants Pioneer 30F53 grown in pots and kept in a greenhouse for 30 days for multiplication. The pre-identification of the specimen was done with semi-permanent blades in formalin, examined under an optical microscope, comparing the characteristics observed with the literature (Handoo and Golden, 1989).

Experimental procedures

The experimental design was completely randomized, in a factorial scheme (2×6), composed of two sources of extracts (leaf and bark) of black angico under six concentrations (0, 20, 40, 60, 80 and 100 g L^{-1}), with five replications per treatment.

The seedlings were prepared in trays of expanded polystyrene with 128 cells, with substrate consisting of sand, manure and earthworm humus (in the same ratio), sterilized by autoclaving, at a temperature of 120°C and pressure of 1.05 kg/cm^2 for 2 h. Transplanting of seedlings was done on the thirteenth day after emergence, and two seedlings were maintained per pot. Thinning was done 28 days after transplanting, keeping a single plant which corresponded to the experimental unit.

Subsequently, at 4 days after transplanting, a suspension with 2000 specimens/juveniles and inoculum eggs was used for inoculation with the aid of a pipette and distributed in three holes of

5.0 cm deep, spaced 2.0 cm from the hypocotyl of cotton plants to facilitate the development of nematode action in the soil.

The botanical material (leaves and bark) of the black angico plant species was collected in the region of Bom Jesus – PI. The dehydration process was done in the laboratory at room temperature during 5 days, then subjected to a mechanical mill pulverization process, reduced to powder, and stored in a 1000 ml beaker until the preparation of the fractionated aqueous extracts.

One day prior to application of the treatments, the bark and leaf powder of Angico at concentrations 0, 20, 40, 60, 80 and 100 g/L was subjected to cold extraction with distilled water for 24 h to obtain the maximum extraction of the chemical constituents. The resulting extractive solution was filtered and then applied in the treatment through the soil.

A solution of 100 ml was applied in each pot. It was divided into 4 aliquots of 25 ml each, at intervals of 15 days. The concentrations used throughout the intervals were prepared only 24 h before the applications.

Analyzed variables

The evaluations were performed sixty days after application of the extracts. Agronomic variables of the cotton plant were evaluated: plant height and root length using a graduated ruler; fresh shoot mass and the fresh root mass, obtained with the aid of a semi-analytical balance. Root volume was measured using a 1000 ml test tube, considering a fixed volume of 800 ml and immersing the root in this volume, calculating the difference to obtain the final volume.

The variables on parasitism were estimation of the number of specimens in the soil of each treatment, extracted in 100 cm^3 of soil by centrifugation and flotation (Jenkins, 1964) and estimation of the root nematodes (Coolen and D'herde, 1972).

Statistical analysis

Data on agronomic variables and parasitism were analyzed by the Shapiro-Wilk test and the analysis of variance (ANOVA) by the F test ($p < 0.05$), using the statistical program "R" version 3.1.2. When significant, the mean were adjusted in regression equations using the software SigmaPlot 10.0.

RESULTS AND DISCUSSION

Influence of aqueous extracts of black angico on cotton plants

By the analysis of variance, it was observed that there was an interaction between sources and concentrations of the black angico extract, with significant effect only for volume ($P< 0.01$) and fresh root mass ($P<0.05$). At the same time, only the plant height ($P<0.01$) was affected by the individual performance of the extract concentrations.

The height of cotton plants was positively influenced by the extracts of black angico. Regardless of the source tested (bark or leaf), there were quadratic responses as a function of the concentrations applied (Figure 1A). Thus, the plants showed greater heights when they received 60.83 g L^{-1} of the extract, reaching an increase of 25.80% (Figure 1B). However, the plants showed a reduction in growth at concentrations above 60.83 g L^{-1}. The harmful effect of the black angico extract on the plant in high concentrations could be related to the presence of tannin in this species, which is considered an allelopathic agent, due to its ability to act directly on the cytological characteristics, phytohormones, membranes, mineral absorption, respiration and enzymatic activity (King and Ambika, 2002).

Root volume and root fresh mass of cotton plants presented positive gains after the extracts application as means adjustment was made in the quadratic polynomial regression model (Figure 1B and C). The highest averages of these variables were observed with a leaf extract concentration of 60 g L^{-1}, reaching respective maximum increases at 30.61 and 15.01%, at the highest concentration tested (100 g L^{-1}). The increase in the root system with leaf extract can be attributed to the reduction of the parasitism of *P. brachyurus* (Figure 2), which is also associated with other factors such as the presence of some allelochemicals stimulating the roots development, as well as an allelopathic effect (Carvalho et al., 2002). Abreu (1997), reported a possible presence of an allelochemical in the aqueous extract of red angico (*Anadenanthera peregrina* (L) Speg), acting as a phytohormone in the roots development.

For the bark extract, the best results for volume and fresh root mass occurred at low concentrations of 37.38 and 30 g L^{-1}, respectively. However, at concentrations above these, there was a reduction of root development due to allelopathic effects demonstrated by the bark extract (Figure 1B and C). As previously mentioned, tannin is the main chemical constituent present in the leaves and bark of black angico, which may interfere with the physiological activity of plants, harming or stimulating growth and development. Thus, the divergent results between leaf and bark extract may be related to the pronounced presence of tannin in the barks (around 15 to 20%) (Lorenzi and Matos, 2002).

Influence of aqueous extracts of black angico on *P. brachyurus* parasitism

For the variables of *P. brachyurus* parasitism, there was no significant interaction ($P>0.05$) between sources and concentrations of black angico extracts (Table 1). However, there was a significant effect ($P<0.01$) of extract concentrations on juvenile variables in the root and juveniles in the soil.

The extracts of black angico influenced negatively the number of juveniles of *P. brachyurus* in the root, with exponential reduction according to the tested concentrations (Figure 2A). These results demonstrate that the lowest applied concentration (20 g L^{-1}), regardless of the source (bark or leaf), was efficient in reducing root nematodes by 71.43%. These results of *P. brachyurus* control may be related to tannins presence in black angico bark (Lorenzi and Matos, 2002), and may present antimicrobial activity (Djipa et al., 2000). Tannins

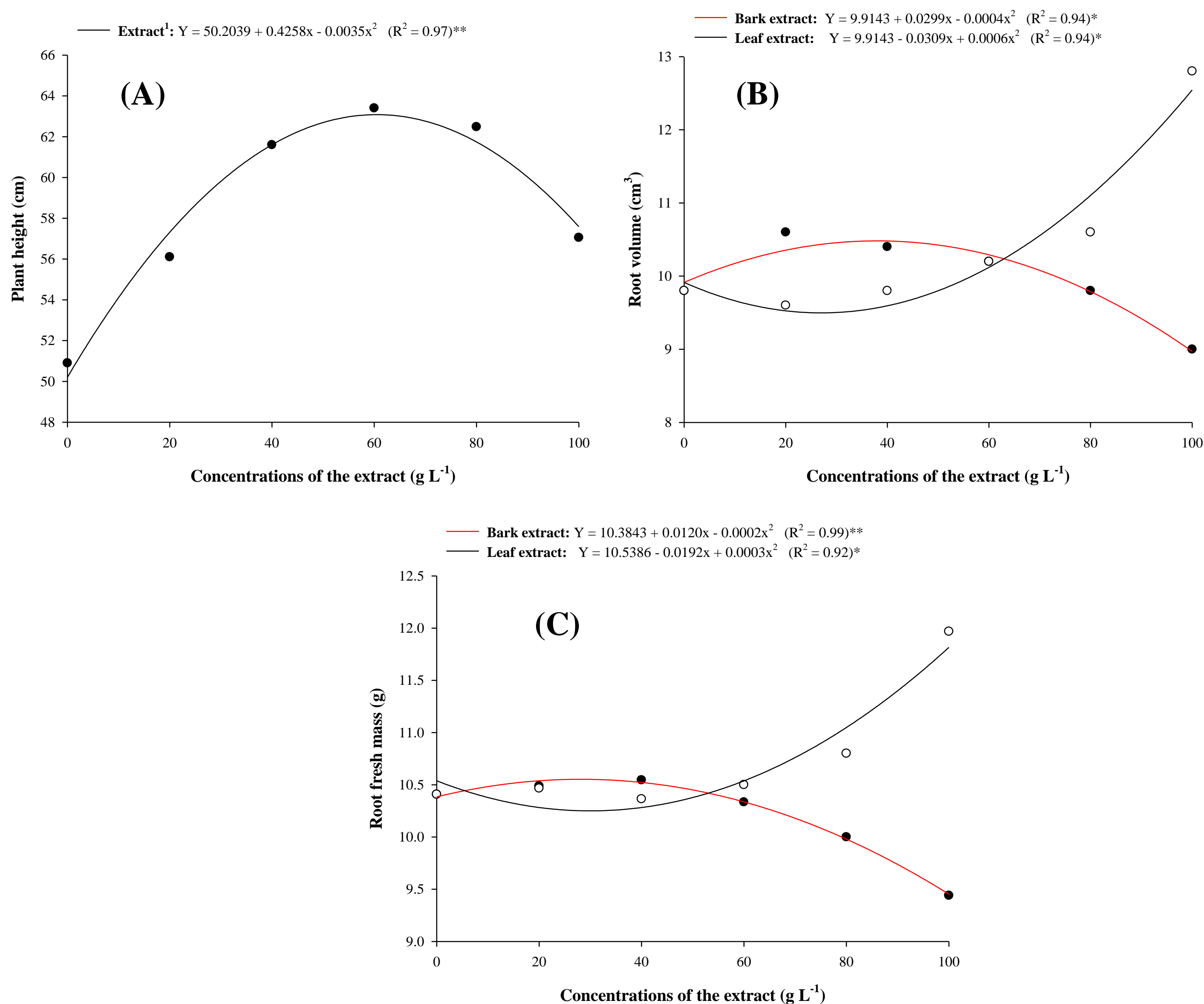

Figure 1. Plant height (A), root volume (B) and root fresh mass (C) of cotton plants according to the concentrations of aqueous extract of black angico. **Significant at 1%; *Significant at 5%. Extract[1] - Individual effect of extract concentrations, independently, from the source tested.

have action on microorganisms' cell membranes and modify their metabolism (Scalbert, 1991). Wilted black angico leaves are popularly toxic and can be used as natural defenses (Silva Filho, 2007).

Leaf and bark extracts of black angico reduced the number of juveniles of *P. brachyurus* in soil, with exponential reduction as a function of the applied concentrations (Figure 1B). The lowest number of nematodes in the soil was observed in the lowest extract concentration applied (20 g L^{-1}), with a reduction of 74.83%. The nematicidal action of black angico is attributed to compounds involved in chemical defense, which include lectins, protease and amylase inhibitors, toxins, and low molecular mass secondary metabolites (Xavier-Filho, 1993). As the extracts in this research were directly applied on the soil, possibly the compounds present in the leaf and bark of the black angico have acted directly by contact on the nematodes, promoting a population decrease. Maistrello et al. (2010), demonstrated the nematicidal action of tannin in preventing hatching and development of phytonematodes of the *Meloidogyne* genus.

Several natural substances of different plant species have been isolated and chemically characterized, and some are promising for field application. Martinez (2002) demonstrated the nematicidal effect of neem on several species of phytonematodes such as *Pratylenchus* species, *R. reniformis*, and *M. incognita.* Franzener et al.

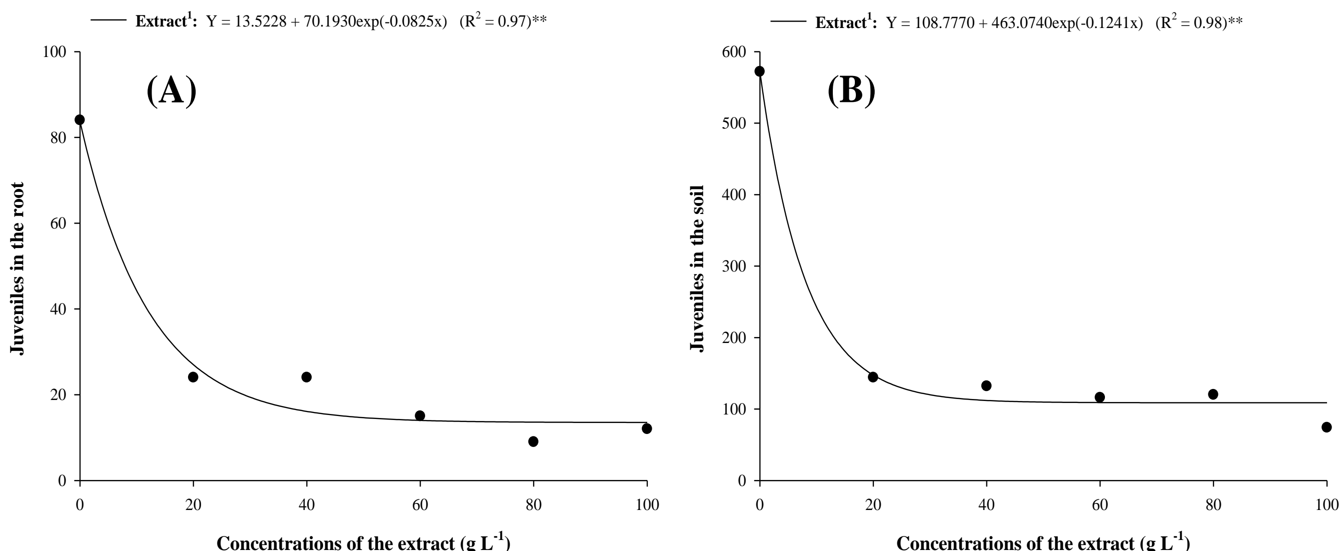

Figure 2. Juveniles in the root (A) and juveniles in the soil (B) of *P. brachyurus* according to the concentrations of black angico extract. **Significant at 1%. Extract[1] - Individual effect of extract concentrations, independently, from the source tested.

Table 1. Summary of variance analysis for cotton agronomic variables: plant height (PH), shoot fresh mass (SFM), root length (RL), root volume (RV) and root fresh mass (RFM) and for the variables of the *P. brachyurus* parasitism: juveniles in the root (JR) and juveniles in the soil (JS).

Source/Variation	Agronomic variables					Parasitism variables	
	PH	SFM	RL	RFM	RV	JR	JS
(ES)[1]	52.82^{ns}	68.84^{ns}	2.32^{ns}	3.22^{ns}	1.35^{ns}	106.66^{ns}	1500^{ns}
(EC)[2]	230.07**	16.91^{ns}	96.57^{ns}	0.61^{ns}	2.35^{ns}	7908**	350364**
ES x EC	51.9^{ns}	50.11^{ns}	7.38^{ns}	5.13*	8.27**	310.66^{ns}	6140^{ns}
CV (%)	8.38	12.74	14.65	11.82	11.46	42.52	33.23

**Significant at 1%; * Significant at 5% and ^{ns}not significant. (ES)[1]: Extract source; EC[2]: Extract concentrations.

(2007) verified the nematicidal effect of the aqueous extract at 0.05 g ml^{-1} of *Tagetes patulae* flowers when applied to the soil, observing a reduction of 62.2, 61.5 and 52.8%, in the number of galls, number of juveniles in the soil and number of eggs for *M. incognita* in tomato roots, respectively. Aqueous extracts obtained from crotalaria leaves (*Crotalaria mucronata* L.), at a concentration of 0.2 g ml^{-1}, when applied via soil in tomato plants, reduced the number of galls caused by *Meloidogyne javanica* by 33% compared to the control, in which only water was applied (Gardiano et al., 2010).

Thus, the use of secondary metabolites of plants with nematicidal properties represents an economically viable option, since it presents a lower risk of environmental contamination due to its biodegradable characteristics. However, it is necessary to carry out new studies to characterize the active ingredients in the extracts so as to pinpoint the mode of action of the extracts on cotton plants. In addition, this could pave the way for possible synthesis of botanical nematicides based on the extracts.

Conclusions

The aqueous extracts of black angico present nematicidal potential and promote plant growth and development.

The leaf aqueous extract contributed to an increase in root volume and root fresh mass of cotton plants.

Leaf and bark extracts of black angico negatively influenced plant height was at concentrations higher than 60.83 g L^{-1}. Root volume and root fresh mass decreased when exposed to concentrations above 37.38 and 30 g L^{-1}, respectively of bark extract.

All concentrations for leaf and bark extracts showed some nematicidal action, mainly in the lower concentrations (20 and 40 g L^{-1}).

Conflict of Interests

The authors have not declared any conflict of interests.

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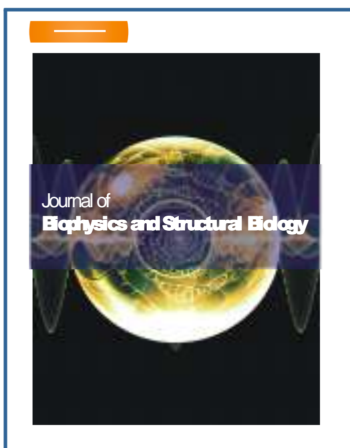

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