# Bioactivities and Cytotoxicity Evaluation of *Actinokineospora fastidiosa* nova strain NAD R4 Extract: Potential for Biomedical and Agricultural Applications

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**ABSTRACT**:

Actinomycetes are gram-positive, filamentous bacteria colonize varied ecosystems. Selection of different natural environments, lik soil, marine water and sedements with diverse climates, enhance chances of discovering novel rare actinomycetes with potentially novel compounds. This study focuses on isolation, purification, identification, and evaluation of bioactive substances produced by rare actinomycetes. Various soil samples from Egypt were examined and the most potent actinomycetes isolate had been identified as Actinokineospora fastidiosa nova strain NAD R4 using phenotypic and genotypic characteristics, following the suggested Bergey's Manual International Keys for actinomycetes identification.The isolate exhibited remarkable bioactivities including antimicrobial, bioherbicidal, biofertilizer properties and the synthesis of tellurium and silver nanoparticles. Optimal conditions for biomass productivity and antimicrobial activity were achieved using sterilized starch-nitrate liquid medium, incubated at 35 °C with a pH of 5, supplemented with starch (3.0 g /100 ml) as the carbon source and L-asparagine (0.25g/100 ml) as the nitrogen source. Incubation has been going on for 7 days with speed of 150 rpm. A solvent containing ethyl acetate was used to extract the antimicrobial compound. Purification using TLC and HPLC, exhibited a potent antimicrobial activity against selected microorganisms. The purified compound was characterized through UV, IR, HNMR, and LC-Mass analysis and identified as a new derivative of izumiphenazine C, with the chemical formula $C_{20}H_{15}N_3O_3$. MIC and MPC of the compound were determined against different tested bacteria. The compound showed low cytotoxicity on the normal cell line of human embryonic kidney (HEK-293), while displaying high toxicity against the human liver cancer cell line (HepG2).

**Keywords:** Rare actinomycetes; *Actinokineospora fastidiosa nova strain* NAD R4*;* bioherbicidal, biofertilizer; antimicrobial agent; Cytotoxicity; izumiphenazine C.

## INTRODUCTION:

Actinomycetes represent a widespread category of prokaryotic microorganisms extensively distributed in the natural surroundings. They exist in various environments and are an important component of microbial diversity (Selim *et al.,* 2021). They grow on solid media with a formation of substrate and aerial mycelium, which culminates in sporulation of spores with different spore surfaces. Usually, they favor low levels of moisture content for growth, survival and possess the capacity for sporulation even in dry environments. They are mesophilic, although certain species could be present in thermal habitats. Additionally, they have a broad range of hosts and can grow as epiphytes (Shanthi, 2021).

Rare actinomycetes are underexplored non-*Streptomycetes* that are less usually isolated than commonly isolated *Streptomyces* spp. This nomenclature is not a reflection of their natural abundance, but rather of the fact that, in comparison to the genus *Streptomyces,* they are less often isolated and have received less research. This is most likely due to their slower growth (in laboratory environments) and the absence of focused isolation techniques (Zamora *et al.*, 2022).

Rare species rather than *Streptomyces* constitute a priceless source of bioactive substances (Rachman and Wibowo, 2024). They are believed to provide novel natural product sources and interestingly new compounds. These microbial metabolites such as antibiotics, insecticides, anticancer and antioxidants will be of interest to humanity to be employed in industry, agriculture, and medicine. The produced bioactive metabolites from uncommon actinomycetes being investigated for novel therapeutic substances that might serve as a solution for current issues with person health, such as the emergence of infectious antibiotic resistance illnesses and metabolic problems (Ezeobiora *et al.,* 2022*).* This present study targets the investigation of the ability of novel rare actinomycetes, isolated from natural habitats to produce novel bioactive compounds as antibiotics, bioherbicides, biofertilizers, biosynthesized nanoparticles and anticancers.

## MATERIALS AND METHODS:

**Sample Collection:**

The specimens had been gathered from various natural soils at different locations of Egypt, such as Matrouh, Suez, South Sainai (ra's sudar) and red sea governorates at depth of 5cm. The samples were air dried and sieved to remove various contaminants then kept in sealed sterile containers until used for isolation at room temperature.

**Pretreatment techniques of the collected samples.**

A variation in pre-treatment techniques is necessary for selective isolation of uncommon actinomycetes. Heat treatments (wet and dry heat), Calcium carbonate and centrifugation were used (Ezeobiora *et al*., 2022).

**Selective isolation and purification of rare actinomycetes**

Rare actinomycetes`s isolation from collected, pretreated specimens processed on a selective medium of starch casein agar (SCA) enhances growth and proliferation of rare actinomycetes (Lee *et al*., 2017) with supplementation of nalidixic acid (50 mg/L) and nystatin (100 mg/L) in order to suppress the growth of Gram-negative bacteria and fungi (Qin *et al*., 2009). Inoculated petri dishes were incubated at 30 °C up to 30 days and a check for colonies would be conducted every day. A chalky, powdery texture and a leathery apperance are the main characteristics of actinomycetes colonies. Various actinomycetes isolates were specifically obtained, streaked onto agar media containing inorganic starch nitrate, and subjected to purification by repeating the process at least three times. The purified isolates were then kept at a temperature of 4 °C for subsequent analysis and characterization.

**Antimicrobial screening of the obtained actinomycetes isolates.**

Five test bacterial strains include gram negative bacteria such as, (*Escherichia coli ATCC 25922, Enterobacter cloacae LMG 2683 Pseudomonas aeruginosa ATCC 27853* and *Proteus vulgarius ATTCC 13315*) and gram positive bacteria (*Bacillus Subtilis ATCC 6633*) in addition to, four test fungal strains (*Candida albicans MTCC 183, Alternaria alternaria Te 19, Aspergillus terrus SQU 14026* and *Fusarium chlamydosporum F25*) were used as a test microorganism to test the antimicrobial activity of the isolated actinomycetes through the use of an agar plug assay (Cork borer method) according to (Cooper, 1963).The appearance of clear inhibition zones are an indication of the existence of biologically active antimicrobial substances.

**Screening of different bioactivities of the most potent actinomycete isolate.**

The actinomycete isolate with the highest antimicrobial activity was selected for more bioactivities investigations as the following:

***Herbicidal activity against some weeds.***

The pure filtrate of the isolate was used to test the herbicidal activity against some weeds such as rya grass (*Lolium multiflorum*), endive (*Cichorium endivia*) and wild radish (*Raphanus raphanistrum*) as common weeds according to (Macias *et al*., 2000). lengths of each's roots and shoots weed seedlings were detected finally as said by (Chung and Miller, 1995).

***Bio fertilizer activity on some Egyptian economic crops.***

Filtrate resulted from submerged fermentation of the isolate was used in bio fertilizer assay through the application on the seeds of bread wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). In vitro, seed germination bioassay test was performed to assess the biological capacity of the isolated actinomycete strain for improving wheat and barley seed germination. After getting surface sterilized by using 0.024% sodium hypochlorite for 2 minutes, the seeds were agitated and completely rinsed with sterile water (Gholami *et al.,* 2009). Surface sterilized seeds were treated with filtrate of the isolated actinomycete strain. Two replications were considered for the experiment, (Control with sterile distilled water). Ten seeds per petri dish of both wheat and barley. Seeds were arranged in an equidistant manner above filter papers in sterilized Petri dishes (Noumavo *et al*., 2013). After seven days, shoot and root lengths of each seedling had been evaluated and recorded (Dicko *et al*., 2018).

***Nanoparticles biosynthesis.***

Bio synthesizing of nanoparticles possess a number of benefits, such as being easily accessible and safe. Additionally, they have a countless application (Abd-Elnaby *et al.,* 2016). In this investigation, we examined the organism's capacity for tellurium and silver nanoparticles biosynthesis as follows:

*Tellurium nanoparticles biosynthesis (Te NP-biosynthesis):*

Potassium tellurite (K2TeO3) was incorporated into the filtrate of the organism then incubated at 30°C for 48h. The reaction

mixture contains a solution of (2 mmol) potassium tellurite plus 1 ml filtrate. Formation of tellurium oxide demonstrated by the existence of black suspended particles, based on a comparison with control samples which contain only potassium tellurite with distilled water (Amoozegar *et al*., 2008 and AboElsoud *et al*., 2018).

*Silver nanoparticles biosynthesis (Ag Nps - biosynthesis):*

By dissolving 0.017 g of silver nitrate in 100 ml of distilled water, a solution of silver nitrate (1 mmol/L) was obtained. After that, 5 milliliters of the actinomycete`s filtrate was mixed with 95 milliliters of silver nitrate solution. Mixture was remained at room temperature in a dark area for the next seven days. The color changing to brown signifies the positive outcomes of AgNps biosynthesis (El-Ghwas and El-Waseif, 2016).

**Identification of the selected most bioactive actinomycete isolate.**

***Morphological, biochemical and physiological identification.***

Actinomycete isolate's morphological and cultural traits were made by media suggested for International *Streptomyces* Project (ISP-media) according to (Pridham *et al.*,1958; Shirling and Gottlieb, 1966). Microscopic studies also were performed by using a cover slip culture teqnique (Kawato and Shinobu, 1959), examined with light microscope (Optika, Italy) with oil immersion Lense x100 and scanning electron microscope took place at Alexandrea University's Faculty of Science utilizing (JEOL Technics, Japan). Cell wall analysis was done utilizing the techniques specified by (Becker *et al.*,1964) and (Lechevalier and Lechevalier, 1970) in addition to different biochemical and physiological tests according to (Holt *et al.,* 1994). Colors were evaluated using the scale created by (Kenneth, 1976).

*Molecular or Phylogenetic identification*

Phylogenetic identification was operated in National Research Center in (Dokki, Giza governorate, Egypt). Genomic DNA of the isolate under study was isolated according to **(Sambrook *et al.,* 1989).** Using universal primers, 16S rRNA gene fragment was amplified via polymerase chain reaction (PCR).

(Actinomytcetes forward specific Primer) with the sequence:

5'-GGATGAGCCCGCGGCCTA -3' and actinomycetes Specific Reverse primer

3'- CCAGCCCCACCTTCGAC -5' (Monciardini *et al.*, 2002). Using the Blast tool (www.ncbi.nlm.gov/blast), the sequence similarities and phylogenetic analysis were ultimately found. Clustal X 2.0.11 was used to align the 16S rRNA gene sequences with reference sequences that were acquired from Gene Bank. (Thompson *et al.*, 1997). Using the neighbor-joining approach, the phylogenetic tree was presented with genius pro 7.1.5 (Saitou and Nei ,1987).

**The effect of optimal cultural conditions on biomass and antimicrobial activity of the most bioactive actinomycete isolate.**

Different factors like the impact of the incubation state, incubation temperatures, incubation periods, pH values, in addition to nitrogen and carbon sources with different concentrations were investigated to detect the optimal growth and antimicrobial bioactivity of the isolate. Once the incubation period had been ended, the culture of each parameter was filtered then centrifuged at 3000 rpm. Using the previously described agar diffusion method, the antibacterial activity of the chosen isolate was evaluated by using the obtained filterate against most susceptibl test microorganisms (Ababutain *et al.,* 2013). The filtered biomass was dried on preweighed filter paper for an entire night at 50 °C in the oven to obtain a consistent dray weight, expressed as g/50 Ml (Abou El-Enain *et al.,* 2023).

**Extraction of the antimicrobial bioactive compound(s) from the selected most bioactive actinomycete isolate.**

The highly active actinomycete isolate was exposed to submerged fermentation. Depending on the outcomes of primary and secondary antimicrobial screening, liquid-liquid extraction with different solvents was done (Rajivgandhi, *et al.*, 2019). Petroleum ether, methanol, ethanol, acetone, ethyl acetate, chloroform, and diethyl ether had been added to the filtrate of the fermented culture separately with vigorous shaking in separating funnel then standing for 30 min was employed. There was a separation of two layers; organic and aqueous layers. Organic layer was collected, the microbial activity against the tested microorganisms was detected with agar well diffusion method. The maximal inhibition was used to determine which solvent would be best for the next extraction. Ultimately, a rotary evaporator (SENECO Technology, Taiwan) was used to evaporate the organic phase's solvent and

finally, the fully dried crude extract was gathered.

**Purification of the antimicrobial compound(s) found in crude extract of the most bioactive selected actinomycete isolate.**

***Thin-layer chromatography (TLC)***

The isolate's crude extract was partially purified using silica gel TLC plates in thin-layer chromatography (Kumar *et al.,* 2018). In summary, three distinct TLC plates were spot-tested with 0.1 mL of crude extract sample. Three distinct solvent systems were used to optimize the spotted plates utilizing the ascending solvent approach; chloroform: methanol (9:1 v/v), dichloromethane: methanol (0.5: 9.5 v/v), and water: tetrahydrofuran: formic acid (5:5:5 drops). The UV lamp Model Spectroline (maximum ultraviolet intensity, USA) was then used to see the plates after they had dried, with wavelength range of 254 to 366 nm.

***HPLC***

HPLC analysis was operated according to (Ludwig *et al.,* 2015) with some modifications. A system (Interchim, France) including; mixing HPLC quaternary pump, PDA-UV-Vis detector 190-840nm, fraction collector, and a sample loading module was used to perform preparatory separations. Interchim Software 5.0 was used for process monitoring and system controlling. The sample was added to the column as a dry load with silica after being dissolved in tetrahydrofuran with DMSO. After wards, the chromatographic separation of filtrate was conducted with silica HP-25.0 gm column. Isocretic elution using dichloromethane. As the mobile phase, methanol was employed. A flow rate of one milliliter per minute was used for the elution. With a detection wavelength of up to 300 nm, the UV spectra of the metabolite were acquired from the detector.

**Identification and characterization of the pure active compound.**

An analysis of the physiochemical properties of the purified substance gotten from the most active actinomycete isolate was conducted. Using UV analysis, 1H NMR spectroscopy, IR spectroscopy, and LC-MS analysis, we evaluated their elemental content, solubility, spectroscopic characteristics, and empirical formula. A study conducted at drug discovery center of Ain Shams University, Egypt.

**Determination of minimum inhibitory concentration (MIC).**

MIC of the purified active compound was measured using the dilution method according to (Zgoda and Porter, 2001). Serial dilutions (100, 50, 25, 12.5, 6.3, 3.1, 1.5, and 0.75 mg/mL) of the test compound were tested against (*Enterobacter cloacae LMG 2683, Escherichia coli ATCC 25922, Proteus vulgarius ATTCC 13315 and Bacillus Subtilis ATCC 6633*). 0.1 mL of each dilution was added to 5 mL of inoculated nutrient broth medium containing 0.05% phenol red. The identical mixture was used in the negative control, but the active ingredient was absent. The process of incubation was done at 37 °C for 24 h. Two runs of the experiment were conducted. The color change was an indication of microbial growth, red in the absence of growth and yellow in the presence of it. MIC defined as the lowest concentration of the compound at which color shift not occurred.

**Determination of minimum bactericidal concentration (MBC)**

It was discovered that the MBC was the lowest concentration that eliminated 99.9% of the bacterial inoculum following a 24-hour incubation period at 37 °C. The methodology of (Ozturk and Ercisli, 2006) With a few adjustments was used. On nutrient agar plates, ten microliters from two dilutions above the tube used for the MIC experiment (MIC value) were spread out. Following an 18–24h of incubation at 37 °C, colonies were counted. A sample's concentration that produces less than 10 colonies was called the MBC value.

**Cytotoxicity assay and anticancer activity of the purified active compound.**

Human embryonic kidney 293 normal cell line (HEK-293) was utilized for the cytotoxicity experiment, and human liver cancer cell line (HepG2) was employed to determine the test compound's anti-cancer efficacy. The two experiments were run using various concentrations, (10000,5000,2500,1250,625,312.5,156.25, and 78.12 ug/ml) by using (MTT protocol) to determine LC50 according to (Park *et al.*,1987; Berridge and Tan ,1993).

**Statistical Analysis.**

Data analysis using statistics was done by One- way ANOVA (Fisher, 1925) and Tukey test (Tukey, 1977) (Post Hoc Significant deference).

(a- a- Non-significant difference, a- b significant difference)

Means with different letters within column are significant difference, $P \leq 0.05$

Means with the same letters within column non-significant difference, $P \leq 0.05$

## RESULTS AND DISCUSSION:

**Selective isolation and purification of rare actinomycetes**

Eight actinomycetes isolates were obtained then purified from the previously pretreated six soil samples then kept at 4 °C for more investigation.

**Screening of antimicrobial activity of the obtained actinomycetes isolates.**

The isolated actinomycetes demonstrated a broad spectrum antibacterial activity against yeast, also gram-negative and gram-positive bacteria but had no antifungal effect against multicellular test fungi as shown in table (1) and figure (1). These results were compatible with (AL-Mahdi *et al.,* 2011; Gulve and Deshmukh, 2012) who reported that the antibacterial activity of some actinomycetes species was more effective than antifungal activity.

**Screening of different bioactivities of the most active actinomycete isolate R4.**

The most bioactive actinomycete isolate R4 which had the highest antimicrobial activity was selected for more bio investigations as the following:

***Herbicidal activity of R4 isolate against some weeds.***

The herbicidal activity of R4 strain was evaluated against several weed species, including *Lolium multiflorum* (Rye grass), *Cichorium endivia* (Endive), and *Raphanus sativus* (Radish). Different concentrations of the isolate's filtrate (25%, 50%, 75%, and 100%) were evaluated through testing their inhibitory effects on germination of seeds and development of seedlings. The results demonstrated that isolate filtrate inhibited seed germination and caused a noticeable reduction in the length of roots and shoots in the weed seedlings. In the case of *Cichorium endivia,* the highest inhibitory effect was observed at the 100% concentration, completely preventing seed germination. Even at lower dilutions, the filtrate showed a strong inhibitory effect on seedling growth with respect to the control.

For *Lolium multiflorum* and *Raphanus sativus*, both the shoot and root lengths of seedlings were markedly diminished in comparison to the control group. Furthermore, extent of inhibition raised with higher concentrations of the filtrate. The results, recorded in table (2) and represented in figures 2a, 2b, and 2c, indicated significant differences with a p-value of $\leq 0.05$.

These inhibitory effects of the metabolites produced by R4 isolate could possibly be explained by the existence of natural phytotoxic compounds within the culture`s broth. These findings align with a study by (El-Sayed *et al.,* 2014) that revealed the bioactive metabolites of *Streptomyces levis* strain LX-65, behaved as a biocontroling agents against weeds such as *Echinochloa crusgalli, Lolium multiflorum,* and *Eruca sativa.*

***Biofertilizer activity of R4 isolate on some Egyptian economic crops.***

The biofertilizer activity of R4 isolate was assessed on two Egyptian economic crops, *Triticum aestivum* (wheat) and *Hordeum vulgare* (barley). A bioassay was conducted using four distinct degrees of concentration (25%, 50%, 75%, and 100%) v/v of the isolate's filtrate. The results showed that treating barley seeds with the isolate's filtrate led to improved seed germination, as well as increased shoot and root lengths of the seedlings, even at higher dilutions of the filtrate. These findings were matching with (Chouyia *et al.,* 2020), who demonstrated presence of positive effects of *Streptomyces roseocinereus* MS1B15 on the growth and growth parameters of barley plants. Regarding wheat seedlings, the treatment with the isolate's filtrate also led to enhancement of shoot and root lengths of the seedlings, but this effect was more prominent at lower concentrations of the filtrate, rather than the higher concentration. These outcomes agreed with a study conducted by (Doolotkeldieva *et al.,* 2015), who reported that treating wheat and soybean seeds with *Streptomyces fumanus* gn-2 before planting in low-fertility soil had beneficial effects on germination rate, seedling growth, and increased resistance to pathogens. The results of the bioassay, recorded in table (3) and depicted in figure 3 (a and b), indicated the positive impact of R4 isolate's filtrate on the growth and development of barley and wheat crops, with varying effects depending on the concentration used.

***Nanoparticles biosynthesis by R4 isolate.***

R4 isolate is capable of biosynthesizing nanoparticles, specifically tellurium nanoparticles (Te NPs) and silver nanoparticles (Ag NPs). In the case of tellurium nanoparticles biosynthesis (Te NP-biosynthesis), the isolate demonstrated the ability to reduce tellurium salt (K2TeO3) to tellurium metal (TeO). This reduction process was evident through formation of black suspended particles, indicating formation of Te NPs. Figure 4 (a) illustrates this phenomenon. These results agree with a study by (Abed *et al.*, 2023) that revealed the ability of *S. graminisoli* for production of Te NPs that effectively combat some blood bacterial infections by acting as antibacterial agents, either alone or in combination with vancomycin. Regarding silver nanoparticles biosynthesis (Ag NPs-biosynthesis), the isolate was able to reduce silver nitrate to silver metal (Ag) in the form of Ag NPs. This was demonstrated by color shift of the initially colorless silver nitrate test solution, as it turns brown after seven days of dark incubation. Figure 4 (b) represents the result. These findings align with those of (Abou El-Enain *et al.*, 2023), who found that *Streptomyces avermatitis* Azhar A.4 had the ability to synthesize bio-Ag NPs that exhibited a highly toxic effect on black cutworm. In summary, R4 isolate has the capacity to biosynthesize tellurium nanoparticles (Te NPs) and silver nanoparticles (Ag NPs), offering potential applications in various fields including antibacterial and insecticidal activities.

**Identification of the selected most bioactive actinomycete isolate R4.**

***Morphological, biochemical and physiological identification.***

The isolate R4 was grown on different ISP media as recorded in table (4). The mature aerial mycelium color of the of the isolate R4 on different ISP media was range from Gray to white and white-creamy to black substrate mycelium. Diffusible pigments were observed in all ISP-media. It ranged from yellow to brownish yellow on different ISP-media. Melanin pigments were produced strongly on ISP6 and ISP7, while it weakly produced in ISP1. The light and electron micrographs, exhibited the isolate`s morphological characteristics. Figures (5a) and (5b) illustrate this results. Data indicated that isolate R4 was non-motile, Gram-positive, spore- forming actinomycete with spore chains of Retinaculiaperti type with hairy surfaced non-flagellated spores. The aerial mycelium was gray with presence of branched substrate mycelium. Cell wall analysis resulted in existence of meso- diaminopimelic acid (Meso-DAP), thus it was identified as uncommon rare actinomycete.

Numerous physiological and biochemical tests were performed on the isolate as recorded in table (4). Based on similarities with the current descriptions of known genera found in Bergey's manuals of determinative and systematic bacteriology, the genus of the purified isolate was determined. (Williams *et al.*, 1989; Holt *et al*, 1994 and Whitman *et al.*, 2012).

***Molecular identification of actinomycete isolate R4.***

Using 16S rRNA sequencing, the molecular identification of R4 isolate was carried out. The emergence of a single band in the results suggested that the recovered RNA was pure. After partially sequencing of the pure band, it was aligned with the public database of the National Center for Biotechnology Information (NCBI) by using the Basic Local Alignment Search Tool (BLAST) to rare actinomycetes *sp.* The isolates' partial 16S rRNA sequencing was recorded in Gene Bank (https://www.ncbi.nlm.nih.gov/nuccore/OQ946608) under accession number (OQ946608).The isolate R4 showed a similarity level of 100% with *Actinokineospora fastidiosa* comb. Nov. NRRLB-16697 as shown in figure (6) and was given the name *Actinokineospora fastidiosa* nova NAD R4 strain according to the presence of some differences in their morphological, physiological, and biochemical characteristics like, spore surface, starch hydrolysis, growth pH range and growth temperature range.

**Optimization of cultural conditions affecting the growth and antimicrobial activities of *Actinokineospora fastidiosa* nova NAD R4.**

Optimization of cultural conditions, enhancing actinomycetes`s growth and antimicrobial activities (Raziq *et al.*, 2020). Before the 1970s, researchers focused on optimizing the fermentation medium to improve the growth rate and antimicrobial activity of *Actinokineospora fastidiosa* nova NAD R4 strain. Figure (7a) demonstrates that both the antimicrobial activity and biomass of the strain significantly increased under shaking conditions compared to static conditions. This indicates that nutrient availability and aeration achieved through shaking are vital for the strain's growth and metabolic activity. The same outcomes were attained by (Ababutain *et al.*, 2013) for some *Streptomyces sp.*, where shaking culture exhibited higher growth rates

and antimicrobial productivity compared to static culture. The incubation period was also investigated to determine the best growth rate and antimicrobial activity of *Actinokineospora fastidiosa* nova NAD R4, figure (7b) shows that the growth and antimicrobial activity increased gradually from the third to the seventh incubation day, but further extension of the incubation period led to a decrease in biomass as well as the antimicrobial activity. The most antimicrobial activity and growth rate were observed on the 7th day of incubation, which aligns with the findings of (Shazia *et al.,* 2013), who found that the maximum antibacterial activity at the 7th day. However, (EL-Naggar *et al.,* 2003) found that the highest productivity of *S. violatus* was obtained after being incubated under static circumstances for four days. The medium's pH is a crucial factor in optimization. *Actinokineospora fastidiosa* nova NAD R4 strain was allowed to grow in different pH ranges, and both biomass and metabolic activity were investigated at each pH value. Figure (7c) indicates that the strain can grow in acidic, neutral, and alkaline conditions, but the maximum growth and antimicrobial activity were obtained under acidic conditions, particularly at pH 5.0. Low growth and antimicrobial activity were observed at neutral and alkaline pH values. These results are similar to (Fahmy, 2020), who found that *Streptomyces sp*. NMF76 was grew in the pH range of 4 to 10, and acidic conditions were needed for the antimicrobial agent's production with maximum activity at pH5. Temperature also significantly influences the growth of actinomycetes and the formation of bioactive secondary metabolites. Results indicate that *Actinokineospora fastidiosa* nova NAD R4 strain is a mesophilic organism, capable of growing within temperature range of 30 to 40°C. However, the optimum incubation temperature for maximum biomass productivity and antibacterial activity was found to be 35°C figure (7d). Growth and antibacterial activity decreased below and above this temperature, which agrees with the results of (Atta *et al.,* 2011) for *Streptomyces crystallinus,* AZ-A151, which produced high levels of Hugromycine-B at 35°C.

The choice of nitrogen and carbon sources also has significant influence on the development of antibiotics by actinomycetes. *Actinokineospora fastidiosa* nova NAD R4 was able to grow using various carbon sources figure (7e). The best carbon source for maximum antimicrobial activity and biomass productivity was found to be starch. This is in line with (Awadalla *et al.,* 2018), who found that *S. longisporoflavus* achieved maximum mycelial dry weight and significant antimicrobial activity when it utilized the starch as a carbon source. However, (Ripa *et al.,* 2009) found that glucose supplementation resulted in increased synthesis levels of antibacterial metabolites by the isolated *Streptomyces* spp. (RUPA-08PR). Different nitrogen sources, including amino acids, organic, and inorganic compounds, were tested to determine the best nitrogen source for *Actinokineospora fastidiosa* nova NAD R4 strain figure (7f). The strain was capable of assimilating and growing using various nitrogen sources, but the maximum biomass and antibacterial activity were obtained with L-asparagine amino acid. The type and form of the nitrogen source added to The cultural media can exert a notable influence on biomass and antibacterial activity. This finding partially aligns with (Theobald *et al.,* 2000), who observed the increasement of antibiotic production in *S. antibioticus* when it utilized lysine amino acid as a nitrogen source. On the other hand, (Singh *et al.,* 2009) found that the optimal organic nitrogen source achived high growth and antibacterial activity by *Streptomyces tanashiensis s*train A2D was soybean meal.

The antimicrobial activity and biomass yield could be change by varying carbon source concentration in the fermentation media. *Actinokineospora fastidiosa* nova NAD R4 strain was capable of assimilating and growing using various starch concentrations. The optimum at which high yield of biomass and antimicrobial activity were obtained with starch concentration of (3.0g /100 mL) as shown in figure (7g). Our results were similar to (Abouzaid, 2015) who found that the best concentration of carbon source for *Streptomyces scobiforms*, FA-25 was 3.0g/100 ml. In contrast to (Ababutain *et al.,* 2013) who showed that the greatest growth and antibiotic generation was achieved by (2.0 g/100 mL) soluble starch supplementation. Also, nitrogen source concentration has a significant impact on antibacterial activity regulation and biomass yield. *Actinokineospora fastidiosa* nova NAD R4 strain showed production of biomass also, antimicrobial activity with various L-Asparagine concentrations. The high yield of biomass and antimicrobial activity were obtained with (0.25) g/100 ml of L-asparagine as shown in figure (7h). This outcome was compatible with those obtained by (Hassan *et al.,* 2001) who reported that the maximum antibiotic production by *Streptomyces violatus*

was obtained in production media supplemented with (0.25) g/100 mL of sodium nitrate.

**Extraction of the active antimicrobial compound(s) from *Actinokineospora fastidiosa* nova NAD R4 strain.**

Extraction process of chemical compounds which have antimicrobial properties with organic solvents are always more effective than water-based extraction techniques (Lima-Filho *et al*., 2002). Various solvents in this study were employed in order to extract the active antimicrobial compound(s); ethyl acetate proved to be the most effective antibiotic extraction solvent. This outcome matches that which was attained by (El-Naggar *et al.,* 2017; Srivastava and Shanmugaiah, 2019) who reported that, ethyl acetate was an extraction solvent that worked effectively with antibiotics produced by actinomycetes.

**Purification and identification of the antimicrobial active compound(s) extracted from *Actinokineospora fastidiosa* nova NAD R4 strain.**

***Thin-layer chromatography (TLC).***

Separation and purification of the antimicrobial crude extract completed with TLC.

TLC plates showed one band for ethyl acetate crude extract of *Actinokineospora fastidiosa* nova NAD R4 with all kinds of the employed solvent systems with rate of flow (Rf) of 0.6, 0.72, and 0.8. This result was similarly closed to which reported with (Ramani and Kumar, 2012) Who found out that antimicrobial substances produced by *Streptomyces spp.* Sh7, were extracted with (Butanol: n-Propanol: Water 40:40:20) solvent system and purified by thin layer chromatography had Rf value at 0.6. In addition to (El-Naggar *et al.,* 2017) who illustrated that, *Streptomyces anulatus* NEAE-94 produced antimicrobial substances which extracted with (ethyl acetate: chloroform) solvent system, in a ratio of 9:1, had Rf=0.8.

***High-performance liquid chromatography (HPLC).***

HPLC was used to further purification of the crude ethyl acetate extract from *Actinokineospora fastidiosa* nova NAD R4. The analytical HPLC used a mobile phase of dichloromethane and methanol at a flow rate of 1 ml/min, gave a distinct peak at 7.567 retention time of minutes as shown in figure (8a).

***Ultraviolet spectroscopy***

Ultraviolet (UV) spectroscopy was performed to examine the UV spectrum of the compound. The spectrum in in figure (8c) displayed a prominent absorption peak at approximately 370 nm, with a shoulder at around 390 nm. This absorption profile closely resembled that of a phenazine derivative isolated from *Streptomyces sp*. FAB-MS marine actinomycete with UV absorption at 368 nm (Pusecker *et al.,* 1997). The absorption at 372 nm is attributed to the extended conjugation of the phenazine ring system, while the shoulder at 390 nm may arise from vibronic transitions. Additionally, there was a broad, lower-intensity peak in the visible region (450-550 nm), which could be attributed to charge transfer transitions.

***Fourier transform infrared spectroscopy (FTIR).***

Functional groups contained in the compound were detected with Fourier-transform infrared (FTIR) analysis as shown in figure (8b). The analysis revealed several distinct absorption peaks. A broad peak at 3441 cm-1 indicated hydrogen-bonded O-H stretching vibrations, suggesting the presence of alcohols, phenols, or carboxylic acids. The sharp peaks at 2925 cm-1 and 2854 cm-1 indicated saturated hydrocarbons, such as alkyl side chains or ring systems. The peak at 1743 cm-1 indicated C=O stretching in esters, lactones, or carboxylic acids. Other peaks at 1625 cm-1, 1583 cm-1, and 1458 cm-1 were attributed to C=O stretching in amides or C=C stretching in olefinic or aromatic structures. Peaks at 1299 cm-1 and 1034 cm-1 suggested the presence of C-O and C-N stretching, respectively, in esters, ethers, or amines.

***Proton Nuclear Magnetic Resonance (1H-NMR- spectrum):***

1H NMR spectrum of the extracted compound from *A. fastidiosa* nova NAD R4 which illustrated in figure (8d) displayed several distinct spin systems. Aromatic proton signals were observed in the range of 7.0-8.5 ppm, specifically a pair of doublets at 7.41 and 7.52 ppm (J = 8.0 Hz), indicating a 1,2-disubstituted benzene ring. Methyl singlets were observed at 0.70, 0.72, 0.82, and 0.85 ppm, likely corresponding to methyl groups on aliphatic side chains or ring systems. Additionally, a strong singlet at 3.89 ppm with an integration of 3H indicated the presence of

a methoxy group, a common feature in Actinomycete-derived secondary metabolites.

***Liquid chromatography–mass spectrometry (LC-Mass):***

LC-Mass analysis in positive ionization mode revealed a peak at 9.07 min (m/z 3132-3134.0000), which could represent the [M+H]+ ion of a major compound in the sample. Another intense peak at 10.11 min (m/z 2043-9464.0000) may correspond to the [M+H]+ ion of a significant compound. In negative ionization mode, an interesting peak at 12.43 min (m/z 339.2987, 340.3437, 341.2788) was observed, potentially representing the [M-H]- ion of a compound with a molecular weight of 340.3056 figure (8e). One possible candidate for this compound could be izumiphenazines, a phenazine derivative antibiotic natural compound known to be produced by certain actinomycetes. Its molecular formula is $C_{20}H_{15}N_3O_3$ with a molecular weight of 344.97 Da, falling within the detected m/z value range.

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the purified active compound.**

MIC is the lowest concentration at which the compound prevents visible growth of the bacteria, while the MBC is the lowest concentration that kills 99.9% of the bacterial inoculum after 24 hours of incubation at 37 °C. The results showed that the MICs concerned with antimicrobial bioactive substance formed by *Actinokinospora fastidiosa* nova NAD R4 against *Enterobacter cloacae* LMG 2683, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, and *Bacillus Subtilis* ATCC 6633 were 3.1, 6.3, 12.5, and 25 mg/mL, in turn. Corresponding MBCs of the compound on the same tested bacteria were 3.1, 6.3, 25, and 25 mg/mL, in turn as shown in table (6). Remarkable results were mentioned by (Kurnianto *et al.,* 2020) as crude extracts from *Streptomyces* isolates, showing inhibitory activities against *E. coli* ATCC 25922 and *P. aeruginosa* Ina CC B52, with MIC and MBC values ranging from 2.5-10 mg/mL and 5-10 mg/mL. In contrast, (Taechowisan *et al.,* 2021) who exhibited that the antibacterial activity of *Streptomyces sp*. WO8f against *B. cereus* ATCC 7064, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *S. aureus* Sp6, and *P. aeruginosa* ATCC 28753, with MICs and MBCs of 32-128 µg/mL and 256-512 µg/mL, respectively. The variations in MICs and MBCs observed among the tested bacteria could be attributed to differences in the composition and structure of their cell walls. Gram-positive bacteria have peptidoglycan polymers on their cell surfaces, allowing antibacterial agents to penetrate more easily. In contrast, Gram-negative bacteria possess an outer membrane made of lipopolysaccharides that serves as a barrier to hydrophobic and hydrophilic compounds having particular molecular weights (Kurnianto *et al.,* 2020).

**Cytotoxicity assay of the purified active compound.**

Cytotoxicity assay of the bioactive compound produced by *Actinokinospora fastidiosa* nova NAD R4 was conducted on normal cell line of human embryonic kidney 293 (HEK-293). When tested against HEK-293 cells at various concentrations (10000, 5000, 2500, 1250, 625, 312.5, 156.25, and 78.12 µg/mL), the purified compound exhibited a toxic effect. IC50 value at which 50% of the cells were affected, was determined to be 999.56 µg/ml. The relationship between cytotoxicity percentage and the concentration of the active compound against HEK-293 cells can be seen in figure (9a).

**Anticancer activity of the purified active compound.**

Human liver cancer cell line (HepG2) was used in this assay with the different concentrations (10000, 5000, 2500, 1250, 625, 312.5, 156.25, and 78.12 µg/mL). The results showed that the cancer cells were significantly affected by the compound, leading to cell circularization and detachment. These results are matching with the results obtained by (Lewis *et al.,* 2007), who exhibited the cytotoxicity of substituted phenazines XR11576 and XR 5944 against human tumor cell lines such as PEO1 ovarian cancer and MDA-MB-231 breast cancer. IC50 value for the purified active compound against HepG2 cells was determined to be 410.79 µg/ml and the relationship between cytotoxicity percentage and the concentration of the active compound against HepG2 cancer cell line was illustrated in figure (9b).

IC50 values of the purified active compound for both of (HEK-293) and (HepG2) were represented in figure (9c) as, the graph shows that the purified active compound has a low toxicity on normal cell line (HEK-293) in comparsion with the high toxicity against cancer cell line (HepG2).

**CONCLUSION**

A new important field of research direction is isolation and identification of promising rare uncommon actinomycetes with biosynthetic ability, possessing a significant role in production of unique secondary metabolites such as antibiotics, bioherbicides, biofertilizers, bio nanoparticles and anticancer compounds that will be of interest to humanity to be used in agriculture and medicine. From the current study, *Actinokinospora fastidiosa* nova strain NAD R4 was found to had antimicrobial, bioherbicidal, biofertilizer, biosynthesis of nanoparticles and anticancer activities. The isolate was grown in well optimized production media then the active antimicrobial compound was extracted by ethyl acetate. Further purification with TLC & HPLC and characterization of the bioactive antibacterial compound by UV, FTIR, HNMR and LC-mass, resulted in obtaining izumiphenazine C derevative compound, has a high degree of similarity with Izumiphenazine C compound produced from *Streptomyces sp.* IFM 11204 with small modifications, suggest could be a derivative, as derivatives are molecules derived from a parent structure by making adding or removing such as a methyl group. MIC and MBC were detected by using different gram- positive and gram- negative test bacteria. In addition to detection of the cytotoxicity of this purified active compound against normal cell line of human embryonic kidney 293 (HEK-293) and anticancer activity against human liver cancer cell line (HepG2) with determination of IC50 in each case. The results finally, showed that the purified active compound had a low toxicity on (HEK-293) and high toxicity against (HepG2).

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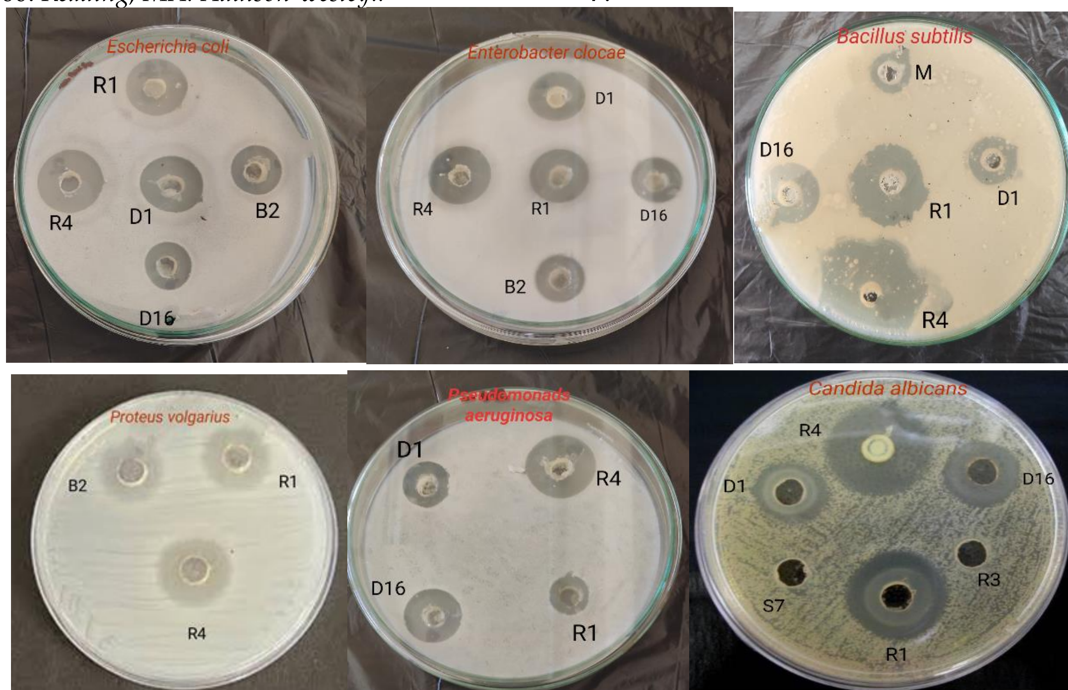
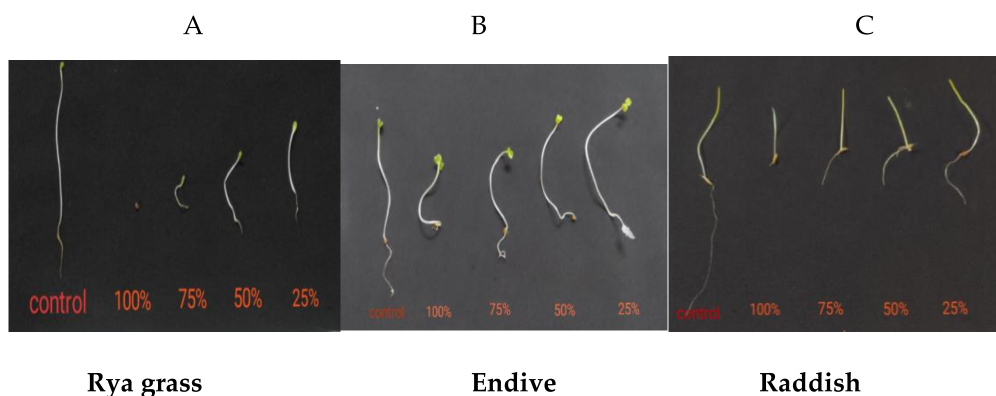



**Figure 1:** Antimicrobial activity of the obtained actinomycetes isolates.

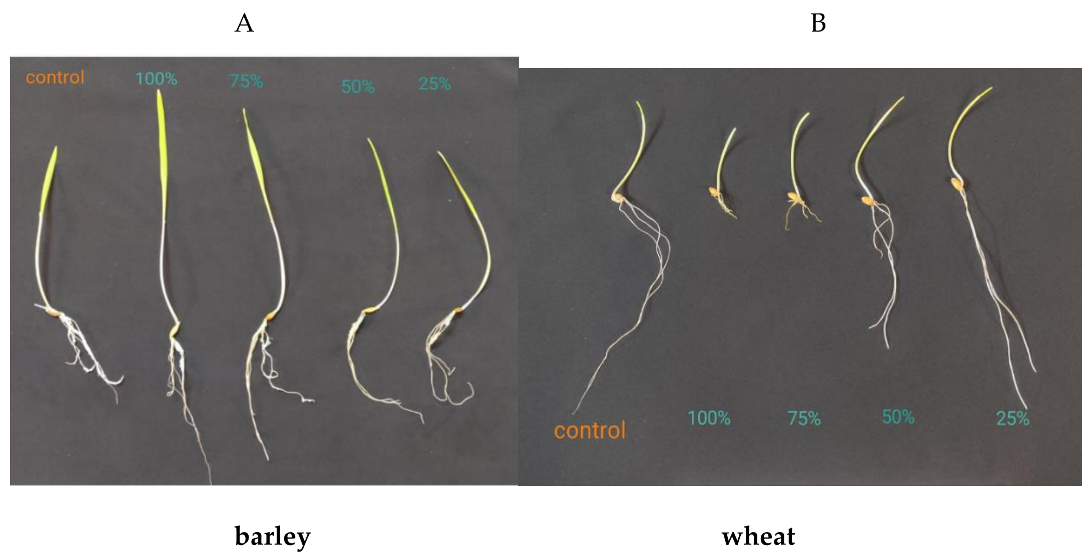
A B C




**Rya grass** **Endive** **Raddish**

**Figure 2:** Bioherbicidal activity of R4 isolate`s filtrate against the seeds of some selected weeds

A B

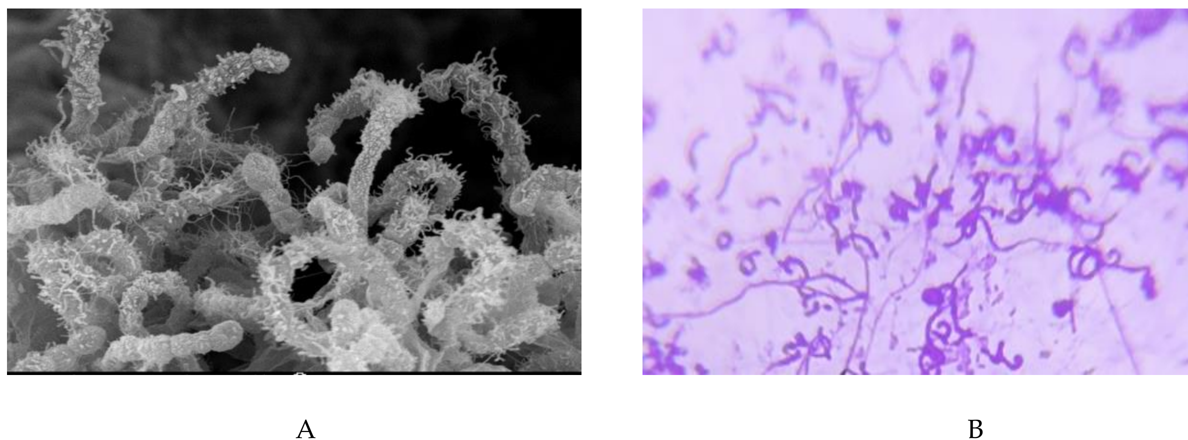



**barley** **wheat**

**Figure 3:** Biofertilizer activity of R4 isolate`s filtrate on barley and wheat.

A B

**Figure 4:** Screening for nanoparticles biosynthesis by R4 isolate (**a**)**:** Te NP- biosynthesis and **(b):** Ag NPs –biosynthesis.



A B

**Figure 5: (a)** Scanning electron micrograph (X10000) showing Retinaculiaperti type shaped aerial mycelia with hairy spore surface of isolate R4, **(b)** The light microscope picture of actinomycete isolate R4 growing on starch nitrate agar media after seven days.

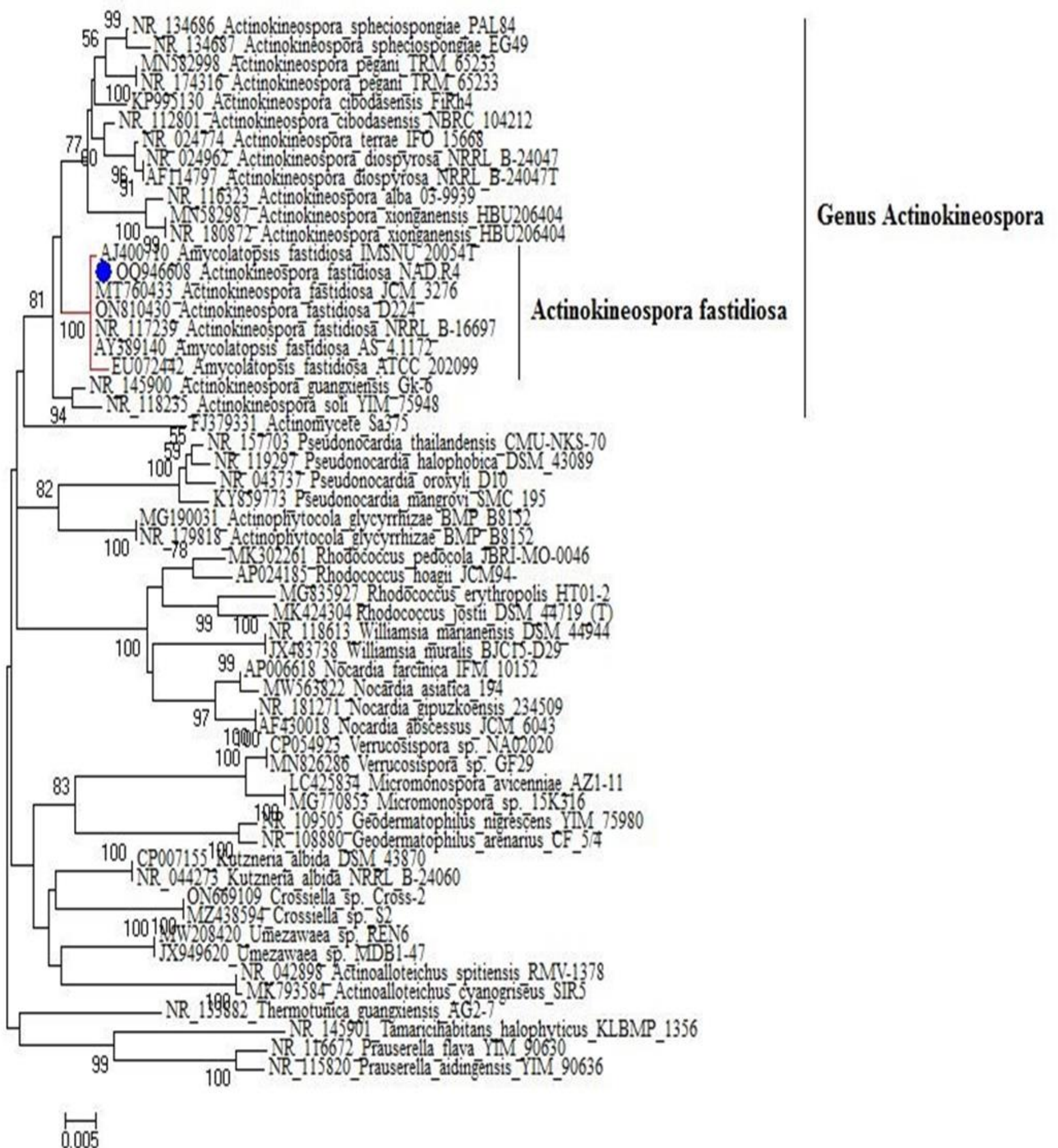
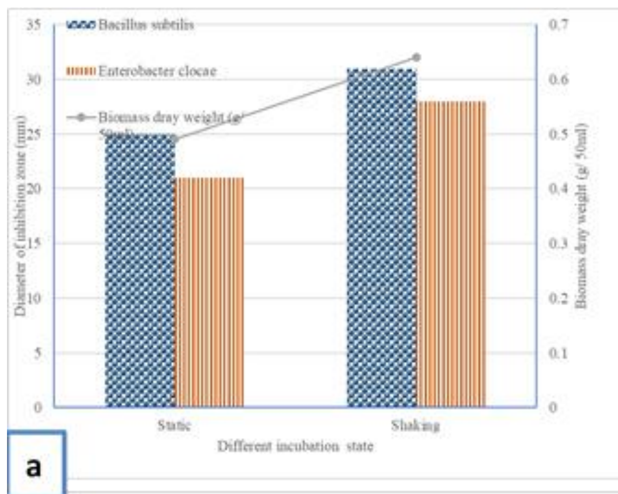
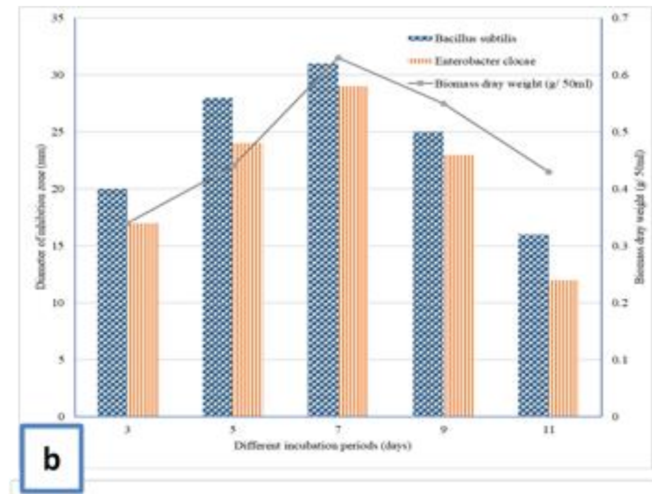
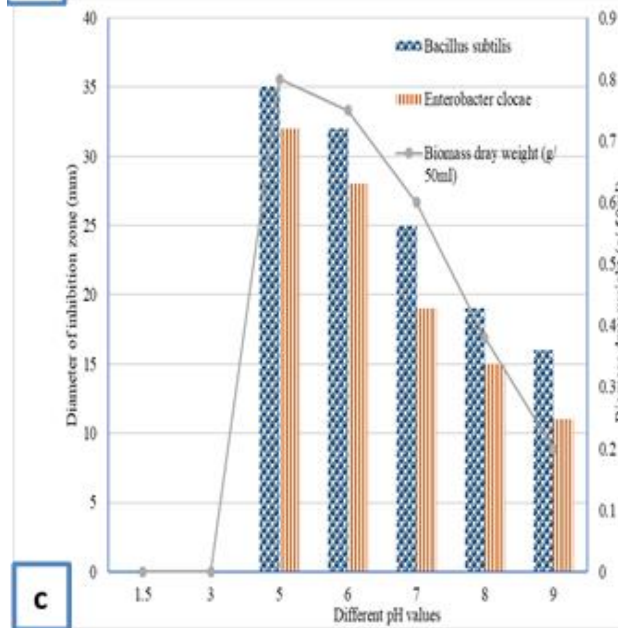
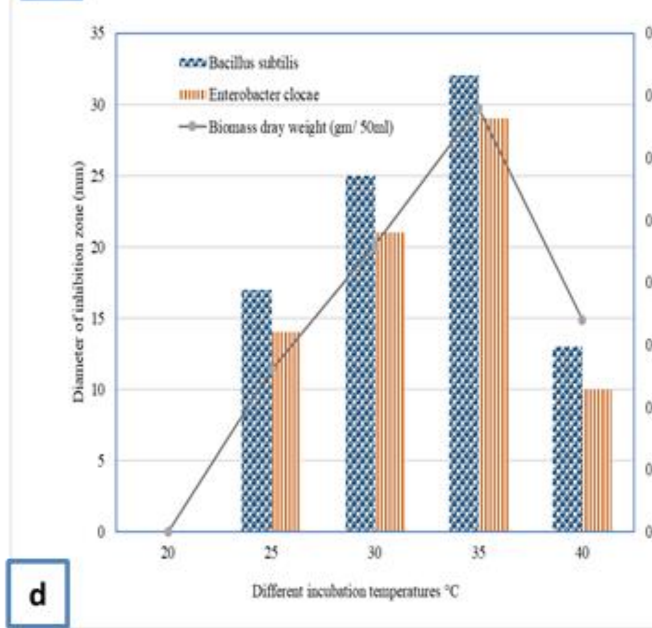


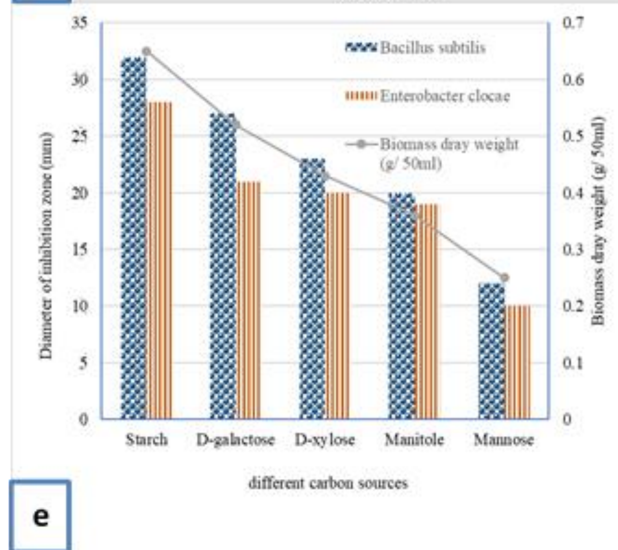

**Figure 6:** Phylogenetic tree of *Actinokineospora fastidiosa* NAD R4 strain and the related species on the basis of the sequencing of 16S rRNA.

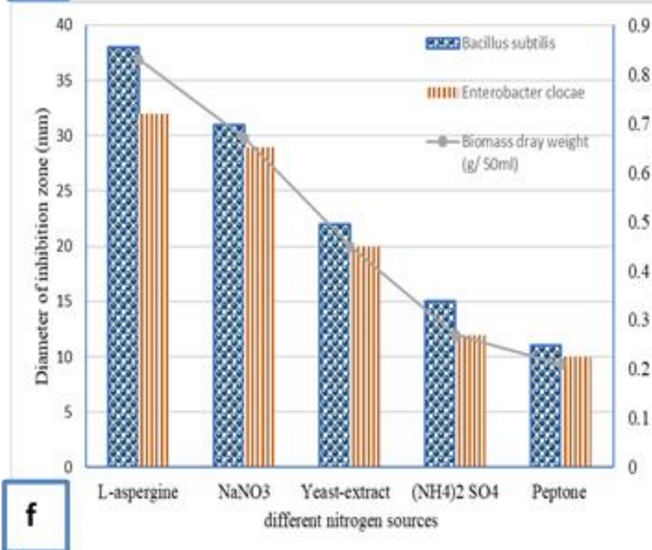

Bacillus subtilis
Enterobacter clocae
Biomass dray weight (g/ 50ml)
Diameter of inhibition zone (mm)
Biomass dray weight (g/ 50ml)
Static
Shaking
Different incubation state
a

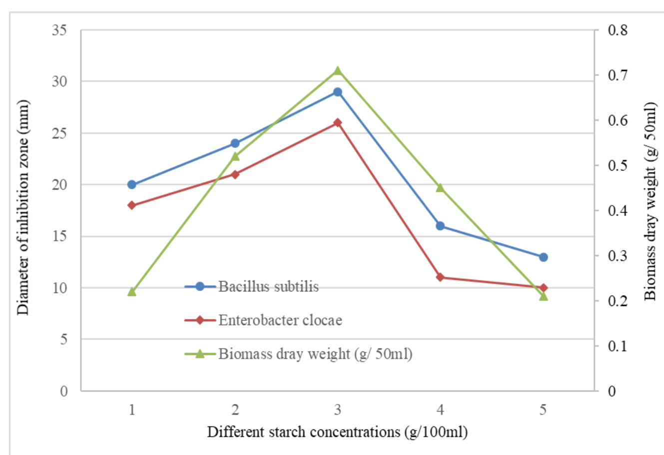

Bacillus subtilis
Enterobacter clocae
Biomass dray weight (g/ 50ml)
Diameter of inhibition zone (mm)
Biomass dray weight (g/ 50ml)
Different incubation periods (days)
b


Bacillus subtilis
Enterobacter clocae
Biomass dray weight (g/ 50ml)
Diameter of inhibition zone (mm)
Biomass dray weight (g/ 50ml)
Different pH values
c

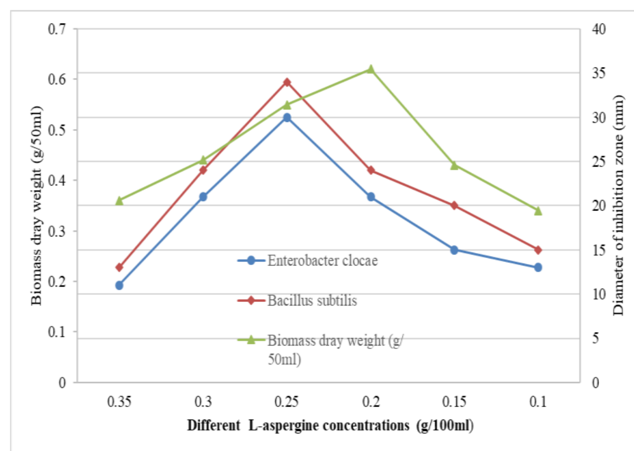

Bacillus subtilis
Enterobacter clocae
Biomass dray weight (gm/ 50ml)
Diameter of inhibition zone (mm)
Biomass dry weight (g/ 50ml)
Different incubation temperatures °C
d


Bacillus subtilis
Enterobacter clocae
Biomass dray weight (g/ 50ml)
Diameter of inhibition zone (mm)
Biomass dray weight (g/ 50ml)
Starch
D-galactose
D-xylose
Manitole
Mannose
different carbon sources
e


Bacillus subtilis
Enterobacter clocae
Biomass dray weight (g/ 50ml)
Diameter of inhibition zone (mm)
Biomass dray weight (g/50ml)
L-aspergine
NaNO3
Yeast-extract
(NH4)2 SO4
Peptone
different nitrogen sources
f




G




H

**Figure 7:** Optimization of cultural conditions that impact the growth and antibacterial activites of *Actinokineospora fastidiosa* nova NAD R4 strain: **a)** different incubation state. **b)** different incubation periods. **c)** different pH values. **d)** different temperature degrees. **e)** different carbon sources. **f)** different nitrogen sources. **g)** different starch concentrations. **h)** different L-asparagine concentrations.

**Figure 8**: Purification and identification of the antimicrobial active compound produced by *Actinokinospora fastidiosa* nova NAD R4 strain: **a)** HPLC. **b)** FTIR **c)** UV. **d)** HNMR. **e)** LC –Mass.

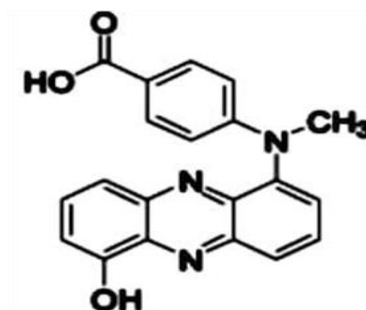



**Figure 7f:** izumiphenazine C

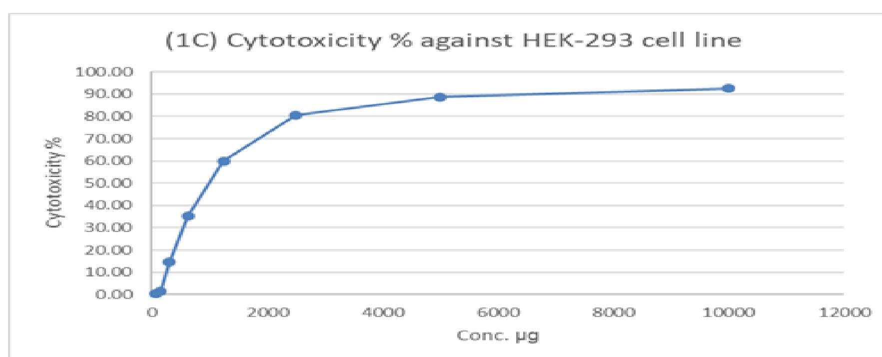



**Figure 9a :**(1C) Cytotoxicity % of the purified compound produced from *Actinokineospora fastidiosa* nova NAD R4 strain against HEK-293 normal cell line.

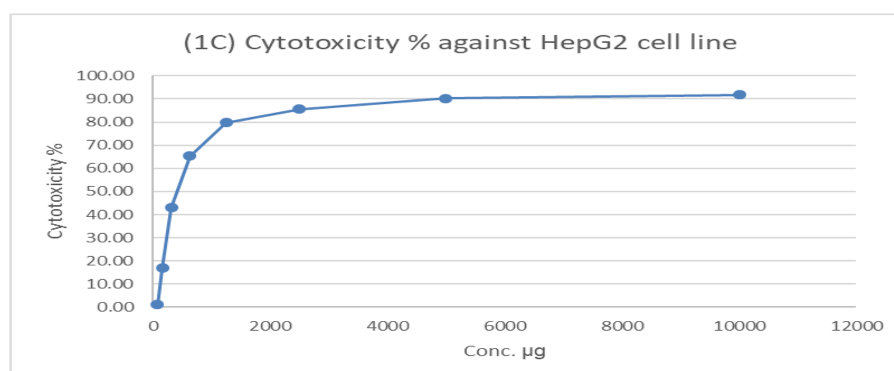



**Figure 9b:** (1C) Cytotoxicity % of the purified compound produced from *Actinokinospora fastidiosa* nova NAD R4 strain against (HepG2) cancer cell line.

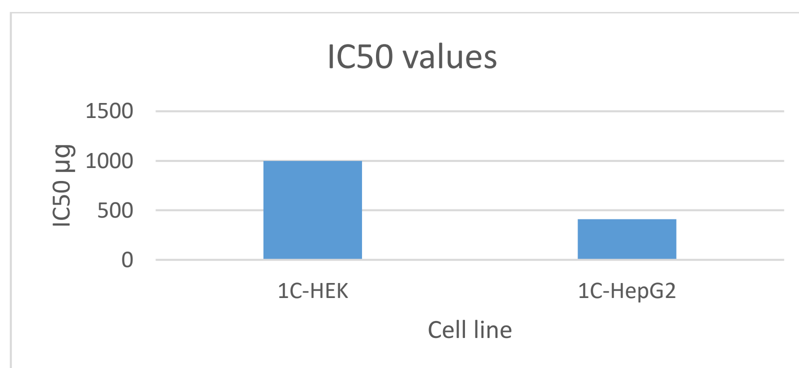



**Figure 9c**: IC50 values of the purified active compound produced from *Actinokinospora fastidiosa* nova NAD R4 strain for both of (HEK-293) and (HepG2).

**Table 1:** Antimicrobial screening of the obtained actinomycetes isolates against tested bacterial and fungal species (Inhibition zone diameter mm).

| Isolates | Diameter of inhibition zones (mm). | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Test bacterial strains | | | | | Yeast | Test fungal strains | | |
| | *Escherichia coli* ATCC 25922 | *Enterobacter cloacae* LMG 2683 | *Bacillus Subtilis* ATCC 6633 | *Pseudomonas aeruginosa* ATCC 27853 | *Proteus vulgarius* ATTCC 13315 | *Candida albicans* MTCC 183 | *Alternaria alternaria* Te 19 | *Aspegillus terrus* SQU 14026 | *Fusarium chlamydo-sporum* F25 |
| B2 | 10±0.2 | 15±0.25 | 20±0.1 | 0±0 | 10±0.2 | 0±0 | 0±0 | 0±0 | 0±0 |
| D1 | 12±0.3 | 15±0.4 | 20±0.2 | 15±0.25 | 0±0 | 10±0.1 | 0±0 | 0±0 | 0±0 |
| D16 | 10±0.5 | 15±0.1 | 22±0.2 | 15±0.4 | 0±0 | 20±0.1 | 0±0 | 0±0 | 0±0 |
| R1 | 20±0.1 | 23±0.4 | 28±0.2 | 13±0.3 | 15±0.5 | 23±0.4 | 0±0 | 0±0 | 0±0 |
| R3 | 0±0 | 0±0 | 15±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| R4 | 20±0.4 | 25±0.2 | 36±0.5 | 22±0.1 | 30±0.3 | 30±0.1 | 0±0 | 0±0 | 0±0 |
| S7 | 0±0 | 0±0 | 15±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| M | 0±0 | 0±0 | 10±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |

**Table 2:** Effect of different concentrations of culture filtrate of R4 isolate on seed germination of *Lolium multiflorum* (Rya grass), *Cichorium endivia* (Endive) and *Raphanus sativus* (Raddish).

| The grass / Different concentrations% | *Lolium multiflorum* (Rya grass) Mean ±S.E | | *Cichorium endivia* (Endive) Mean ±S.E | | *Raphanus sativus* (Raddish). Mean ±S.E | |
|---|---|---|---|---|---|---|
| | Root length (df=4,45) | Shoot length (df=4,45) | Root length (df=4,45) | Shoot length (df=4,45) | Root length (df=4,45) | Shoot length (df=4,45) |
| 100 | 0.10±0.01e | 2.78±0. 06e | 0.00±0.00 c | 0.00±0.00e | 0.19±0.03 d | 2.95±0.07 d |
| 75 | 0.88±0.04d | 4.71±0.07d | 0.02±0.01 c | 1.35±0.08d | 0.42±0.07 c | 3.71±0.22 a |
| 50 | 2.48±0.04c | 5.03±0.06c | 0.48±0.04 b | 2.43±0.12c | 0.51±0.08 c | 4.34±0.10 b |
| 25 | 3.04±0.05b | 5.99±0.05b | 0.38±0.09 b | 3.29±0.12b | 1.03±0.04 b | 4.98±0.08 a |
| Control | 4.27±0.04a | 7.32±0.05a | 1.50±0.01a | 6.02±0.02a | 2.02±0.02 a | 5.03±0.02 a |
| F value | 820.56*** | 794.65*** | 160.20*** | 732.71*** | 177.88*** | 52.26*** |
| P | 0.000 | 0.000 | 0.000 | 0.0000 | 0.000 | 0.0000 |
| L.S.D. | 0.23±0.08 | 0.24±0.08 | 0.19±0.07 | 0.33±0.12 | 0.22±0.08 | 0.49±0.17 |

a- non-significant difference, a- b significant difference), Means with different letters within column are significant difference, $P \leq 0.05$ and Means with the same letters within column non-significant difference, $P \leq 0.05$.
S.E: standard error. df: degree of freedom. L.S.D: least significant difference.

**Table 3:** Effect of different concentrations of culture filtrate of R4 isolate on seed germination of *Hordeum vulgare* (barley) and *Triticum aestivum* (wheat).

| The plant / Different Concentrations % | *Hordeum vulgare* (barley) Mean ± S.E. | | *Triticum aestivum* (wheat) Mean ± S.E. | |
|---|---|---|---|---|
| | Root length (df=4,45) | Shoot length (df=4,45) | Root length (df=4,45) | Shoot length (df=4,45) |
| 100 | 12.12±0.06a | 16.13±0.04a | 1.96±0.05e | 2.97±0.05d |
| 75 | 10.12±0.05b | 15.10±0.04b | 2.23±0.05d | 3.44±0.08c |
| 50 | 9.08±0.03c | 13.30±0.07c | 7.05±0.03c | 6.17±0.08b |
| 25 | 8.36±0.06d | 12.18±0.07d | 11.11±0.07a | 7.25±0.09a |
| Control | 7.02±0.03e | 11.01±0.03e | 10.00±0.03b | 6.01±0.03b |
| $F_{(4,45)}$ value | 1645.89*** | 1480.46*** | 7436.322*** | 728.878*** |
| P | 0.000 | 0.000 | 0.000 | 0.0000 |
| L.S.D. | 0.19±0.07 | 0.22±0.08 | 0.19±0.06 | 0.27±0.09 |

(a-a Non-significant difference, a- b significant difference), Means with different letters within column are significant difference, P ≤ 0.05 and Means with the same letters within column Non-significant difference, P ≤ 0.05.

S.E: standard error. df: degree of freedom. L.S.D: least significant difference.

**Table 4:** Cultural characteristics of actinomycete isolate R4, grown in different ISP media.

| Type of media | Growth | Color of aerial mycelium | Color of substrate mycelium | Color of diffusible pigments |
|---|---|---|---|---|
| Tryptone yeast extract broth (ISP-1) | Moderet | Whitish Gray | Whitish brown | Yellowish brown |
| yeast extract-malt extract agar (ISP2) | Weak | Light gray | White | Strong yellow |
| Oatmeal agar (ISP-3) | Good | Gray | White | Pale Yellow |
| In organic- trace salt- starch agar (ISP-4) | Good | Gray | White | Yellow |
| Glycerol asparagine agar (ISP-5) | Moderate | Gray | White | Yellow |
| Peptone yeast extract iron agar (ISP-6) | Weak | Whitish Gray | Blake | Yellowish brown |
| Tyrosine agar (ISP-7) | Good | white | White | Brown |

**Table 5:** Biochemical and physiological characteristics of actinomycete isolate R4.

| Characteristics | Results | Characteristics | Results |
|---|---|---|---|
| Diaminopimelic acid (DAP) detection:<br>Nitrogen sources utilization:<br>L- asparagine<br>L-tyrosine<br>L- cysteine<br>L- methionine<br>Histidine<br>Serine<br>Ammonium sulphate<br>Sodium nitrate<br>Yeast extract<br>peptone<br>Urea | Meso-DAP<br>+++<br>++<br>++<br>++<br>+<br>+<br>+++<br>+++<br>++<br>++<br>–<br>–<br>– | Growth temperature °C:<br>15-20<br>25- 35<br>35-40<br>40-45<br>Growth pH:<br>3 - 5<br>6-9<br>10-12<br>13 | –<br>+++<br>+<br>–<br>++<br>+++<br>++<br>– |
| Carbon sources utilization:<br>D- glucose<br>D-arabinose<br>D-xylose<br>D- galactose<br>Sucrose<br>Fructose<br>Mannose<br>Mannitol<br>Raffinose<br>Cellulose<br>Starch<br>Sensitivity to antibiotics:<br>Amikacin (30 µg/ml)<br>Cefuroxime (30 µg /ml)<br>Imipenem (10 µg /ml)<br>Ceftazidime (30 µg /ml)<br>Sulfamethoxazole (25 µg /ml)<br>Trimethoprim (25 µg /ml)<br>Motility test: | +<br>++<br>+++<br>+++<br>+++<br>+<br>+++<br>+++<br>++<br>+++<br>+++<br>–<br>+++<br>++<br>+++<br>–<br>+++<br>– | Growth in presence of different NaCl con. (%):<br>0.5-3<br>4-6<br>7-9<br>10-12<br>Enzymatic activity:<br>Amylase<br>Catalase<br>Urease<br>Cellulase<br>Citrate utilization.<br>Gelatin liquification<br>Potassium cyanide (KCN)<br>Hydrogen sulfide (H2S) | +++<br>++<br>–<br>–<br>++<br>++<br>–<br>++<br>+<br>+<br>+<br>– |

**Note:** (–): no growth, (+++): good growth, (++): moderate growth, (+): weak growth.

# تقييم النشاطات البيولوجية والسمية لمستخلص من:*Actinokineospora fastidiosa* NAD R4 nova strain مع إمكانية التطبيقات البيولوجية الطبية والزراعية

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**الملخص العربي:**

تركز هذه الدراسة على عزل وتنقية وتحديد وتقييم المواد الحيوية النشطة التي تنتجها الأكتينوميستات النادرة. وقد تم فحص عينات مختلفة من التربة المصرية وتم تحديد أقوى العزلات والمعنية باسم *Actinokineospora fastidiosa* nova strain NAD R4 حيث تم تعريف العزلة باستخدام خصائص الشكل الظاهرى و التعريف الجينى ومن ثم استخدام دليل بيرجى الدولي لمفاتيح التعرف على الأكتينوميستات. وأظهرت العزلة أنشطة بيولوجية ملحوظة، بما في ذلك الخصائص المضادة للميكروبات، والمبيدات البيولوجية، والخواص المخصبة الحيوية، وتخليق جزيئات التيلوريم والفضة النانوية. وقد تم تحسين الظروف المثلى لإنتاج الكتلة الأحيائية والنشاط المضاد للميكروبات باستخدام وسط غذائى من نترات النشا السائل المعقم والذي تم احتضانه عند 35 درجة مئوية مع درجة حموضة تبلغ 5 حيث يحتوى الوسط على النشا بتركيز3 ٪ كمصدر كربونى ، وأيضا.L-asparagine بتركيز0.25% كمصدرنيتروجينى. وتم الوضع على حضان هزاز عند 150 دورة في الدقيقة لمدة 7 أيام مستمرة. تم استخدام مذيب يحتوي على إيثيل استيتات لاستخراج المركب المضاد للميكروبات. وقد أظهرت التنقية باستخدام TLC و HPLCنشاطاً قوياً للمركب ضد الكائنات الدقيقة المختارة. وتم تحديد المركب المنقى من خلال تحليل الأشعة فوق البنفسجية، IR ، وHNMR، و LC-Massكمشتقة جديدة من الإيزوميفينازين C ، مع الصيغة الكيميائية C20H15N3O3 .وقد تم تحديد MIC و MPC للمركب في ضوء مختلف البكتيريا المختبرة. وأظهر المركب سمية منخفضة على الخلايا الطبيعية لكلية الأجنة البشرية(HEK-293) ، في حين أظهر سمية عالية ضد خلايا سرطان الكبد البشري(HepG2) .

**الكلمات الاسترشادية:** الأكتينوميستات النادرة، Actoinokineospora *fastidiosa nova strain* NAD R4، المبيدات الحيوية، التسميد الحيوى، العامل المضاد للميكروبات، السمية. الإيزوميفينازينC .