Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 24(2) May-August 2026]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

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PANTNAGAR JOURNAL OF RESEARCH

Vol. 24(2) May-August, 2026

CONTENTS

AI and geospatial innovations for Indian agriculture: Applications, policy support and emerging research frontiers (Review) **233-247**
ABHISHEK DANODIA, SURESH KUMAR and AJEET SINGH NAIN

Optimizing nitrogen use efficiency and grain quality in basmati rice through Integrated weed management: Insights into nutrient partitioning and Crop–weed competition **248-255**
ROMAN NISSAR, MANZOOR A GANAI, MEHRAJ UD DIN SOFI, ASHAQ HUSSAIN and F. A. BAHAR

Performance evaluation of brown top millet *(Brachiaria ramosa* l.) genotypes under rainfed upland ecology of the Bastar plateau of Chhattisgarh **256-266**
PRAFULL KUMAR

Effect of pre-harvest sprays of nanofertilizers on fruit quality in litchi (*Litchi chinensis Sonn.*) cv. Rose Scented **267-274**
NITIN PANWAR, RATNA RAI and RAJEEW KUMAR

Ethrel induced ripening optimally modulates physicochemical quality, antioxidant properties and shelf life of sapota (*Manilkara zapota* L.) cv. Cricket Ball **275-285**
ASHOK KUMAR SINGH, KIRTI CHETRI, RISHABH RAJ, S. K. GURU and N. C. SHAHI

Influence of drip irrigation and mulching practices on fruit set, yield and water use efficiency of litchi cv. rose scented in the Tarai region of Uttarakhand **286-292**
SHANKAR LAL, N. SINGH, N.K. BIND, PRERNA PRACHI, RAJSHREE, RIYA THAPA and AKHIL RUHELA

Spatial distribution and survey of soybean anthracnose in the Kumaon region of Uttarakhand, India **293-300**
MANISHA DEV and K.P. SINGH

Dimensionality reduction of part milk yield in crossbred cattle using Principal Component Analysis **301-307**
SIMRAN KAUR, ASHIS KUMAR GHOSH, OLYMPICA SARMA and RAVINDER SINGH BARWAL

Comparative histomorphological and histochemical studies on the abomasum of adult Bakerwali goats, non-descript goats and non-descript sheep of the Jammu region **308-318**
JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA

Gross morphology and biometry on the abomasum of adult Bakerwali goat, non-descript goats and non-descript sheep of Jammu region: A comparative study **319-325**
JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA

Arterial blood gas analysis in bovine pneumonia: Comparative study of healthy and diseased cattle **326-331**
ASMITA NARANG and CHARANJIT SINGH

Assessment of commonly used insecticide residues using gas chromatography in buffalo liver samples collected from North India **332-339**
S. K. JAIN, VINOD KUMAR, VINAY KANT, PREETI BAGRI, ARCHANA LOHIYA, GAURI CHANDRATRE and GAURAV GUPTA

***In silico* molecular docking analysis of squalene with major ageing-related molecular targets: matrix metalloproteinase-1, matrix metalloproteinase-9, cyclooxygenase-2, Tumor Necrosis Factor-α, hyaluronidase-1 and B-cell lymphoma-2** **340-352**
PAWANESH TAMTA, KULDIP CHANDRA VERMA and APARNA DIXIT

Characterization of agro-waste (*Parthenium hysterophorus*) derived Carbon Dots and to assess their potential as a sustainable adsorbent **353-360**
NISHTHA NAUDIYAL and R.K. SRIVASTAVA

Assessment of the antibacterial efficacy of endophytic bacteria in combination with neem leaf powder against bacterial pathogens **361-370**
SHIVANI SINGH, AJAY VEER SINGH and VELLAICHAMY MAGESHWARAN

Exploration of endophytic bacterial isolates from Kasni leaves (*Cichorium intybus*) for Phyto-hormone and Phyto-stimulating metabolite production **371-382**
VARSHA MISHRA, SEEMA BISHT, DIVYA PANT, ANUPAMA PANDEY and LAKSHMI TEWARI

Mitigation of dexamethasone-induced cardiac oxidative damage by *Moringa oleifera* and Vitamin E-Selenium: A biochemical study in broilers **383-395**
LATA PALIWAL, SONU AMBWANI and TANUJ KUMAR AMBWANI

Adaptive Neural Networks for dynamic prioritization in autonomous vehicles for road users **396-403**
MUMUKSHU BHATT, SUBODH PRASAD, RAJESH SINGH, ASHOK KUMAR and POOJA TAMTA

Assessing the impact of Geopolitical Disturbances on India's agricultural trade performance **404-409**
JYOTI, SUMAN GHALAWAT, MEGHA GOYAL, SUNIL KUMAR, AMITA GIRDHAR and SUBODH AGARWAL

Black soybean (*Glycine max* (L.) Merill): Nutritional and functional analysis of whole seed, dehusked seed and husk fractions **410-421**
RITA SINGH RAGHUVANSHI, GUNJAN TEWARI, AABHA RAWAT and APURVA

***In silico* bioprospecting of bioactive phytochemicals with potential protective effects against exercise-induced muscle stiffness** **422-433**
NAGARJUN G. S., PAL MURUGAN M., KRISHNANDU MONDAL, PADMASHREE A., WADIKAR D. D. and KUMAR R.

Assessment of the antibacterial efficacy of endophytic bacteria in combination with neem leaf powder against bacterial pathogens

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https://doi.org/10.67063/pjr.vol.24.issue.02.15

ABSTRACT: *Azadirachta indica* A. Juss (Neem) is a divine tree with diverse therapeutic properties. This study aims to evaluate the antibacterial potential of neem-derived endophytic bacteria against pathogenic bacteria in the presence of neem leaf powder. In this experiment, three endophytic bacteria, namely *Bacillus weidmannii* DNS-13, *B. stercoris* DNS-14 and *B. albus* DNS-16, were evaluated for antibacterial activity against pathogenic *Staphylococcus aureus* and *Escherichia coli* by adding different concentrations of neem leaf powder (0.1, 0.5, and 1 % w/v) using the agar well diffusion method. The results revealed that all endophytic bacteria exhibited larger zones of inhibition against *S. aureus* than *E. coli.* Further, supplementation of neem leaf powder delayed metabolite production, but the antibacterial activity was higher and more consistent than non-supplemented treatments. Among the three strains, DNS-14 showed the highest zone of inhibition (21 mm) in the presence of 1% neem leaf powder and maintained antibacterial consistency up to 96 h of duration. The present findings highlight that amendment of neem leaf powder in the culture medium enhances the antimicrobial activity of neem-derived endophytic bacteria against pathogenic bacteria.

Keywords: Antibacterial activity, Endophytes, Neem leaf powder, Pathogenic microbes,

Unregulated use of antimicrobial agents such as antibiotics leads to the emergence of antimicrobial-resistant pathogenic strains, which pose a serious threat to public health. The *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species (ESKAPE) pathogens are a group of clinically important bacteria which show rise in resistance towards antimicrobial agents (Dashti *et al*., 2026). Nunez- Serrano *et al*. (2026) reported that, along with ESKAPE some more microbes are showing a resistance profile, such as *Escherichia coli* and *Candida albicans.*

To overcome the use of less effective antimicrobial agents, alternative, sustainable, novel and natural sources of antimicrobial agents are in focus, particularly that have been obtained from medicinal plants**.** Among medicinal plants, *Azadirachta indica* (Neem) has been used from ancient times in the traditional medicine system, including Ayurveda, Siddha, Unani, Homeopathy, and Modern medicine. Neem is a tropical and sub-tropical slow-growing plant that constitutes more than 300 bioactive metabolites, namely azadirachitin, nimbin, nimbidine, salanin, nimbidol, gedunin, quercetin, etc. (Rao, 1986; Sairam *et al*., 2000; Ali *et al*., 2021)**.** They are structurally complex compounds that exhibit antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, anti-gastric, anti-arthritic, and anti-inflammatory (Subapriya and Nagini, 2005; Gupta *et al*., 2019). One of the important properties of the neem-derived compound is its low toxicity behavior towards warm-blooded animals like humans (Murugesh Babu and Ravindra, 2014). Direct extraction of biologically active compounds from the plant leads to destructive harvesting and over-exploitation of this plant (Mishra *et al*., 2021). Medicinal plants are not only a rich source of bioactive compounds but also harbor diverse communities of microorganisms inside the plant tissue 'endophytes'. Endophytes are the microorganisms that reside inside the plant tissue without causing any harmful effects to the plant (Mageshwaran *et al*., 2022). Endophytes receive protection, nutrition, and a suitable niche for

survival. In return, endophytes contribute to plant fitness by providing growth-promoting traits, solubilizing nutrients, protection against plant pathogens, and abiotic stresses. This symbiotic association gained attention because endophytes can produce bioactive compounds similar to or distinct from their host plant. These compounds, produced by endophytic microbes, are an alternative source of therapeutic compounds and antimicrobial agents (Hassan *et al*., 2017; Duhan *et al*., 2020; Bundela *et al*., 2023).

The present study aims to evaluate the stem-derived neem endophytic bacteria on antibacterial potential against pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli,* in the presence of neem leaf powder supplemented in the culture medium.

MATERIALS AND METHODS

Bacterial culture revival and maintenance

Three stem-derived neem endophytic bacterial isolates *Bacillus weidmannii* DNS-13 (PV802639), *B. stercoris* DNS-14 (PV802640), and *B. albus* DNS-16 (PV802641) were obtained from the Department of Microbiology Culture Collection, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. All cultures were revived on nutrient agar medium at 28±2°C.

Test Bacteria

The antibacterial activity of DNS-13, DNS-14, and DNS-16 strains was evaluated against two pathogenic bacteria, namely *Escherichia coli* ATCC 25922 collected from the Microbial Technology Laboratory, ICAR-NBAIM, Mau, and *Staphylococcus aureus* (CP150127) from the Department of Veterinary Microbiology, GBPUA&T, Pantnagar. All the cultures used in this study were revived on the nutrient agar medium at 28±2°C. All the cultures were stored in 20 % glycerol stock in -20^{0}C for long-term storage.

Preparation of dry neem leaf powder and confirmation of its sterility

Neem leaves were collected from the plant and washed with tap water to remove all the attached dirt. Then the leaves were dried under shade and ground into a fine powder. After grinding, the fine powder was passed through a sieve with a mesh size (75µm) to obtain a uniform particle size. The neem powder was further sterilized by autoclaving at 121°C and 15 psi for 20 min (Khurshid *et al*., 2015). The 0.1%, 0.5% and 1% of dried neem leaf powder was supplemented in the different nutrient broth and autoclaved at 121°C and 15 psi for 20 min. After autoclaving, the broth was observed for the next seven days to check microbial growth. This sterile neem culture-free broth was used as control medium for their respective treatments.

Evaluation of the antimicrobial activity

The experiment was designed to evaluate the antimicrobial activity of endophytic bacteria in the presence of different concentrations of neem powder. Each endophytic strain was inoculated individually in nutrient broth supplemented separately with 0.1%, 0.5% and 1% (w/v) neem powder in a 250 ml Erlenmeyer flask. For each neem concentration, a sterile neem supplemented nutrient broth was maintained as a control. The design of the experiment is presented in Table 1. All the inoculated flasks were incubated in a B.O.D. incubator shaker at 28°C±2°C with continuous shaking at 120 rpm for 4 days. During the incubation period, samples were collected at regular intervals of 12 h to check the antimicrobial activity against the selected bacterial pathogens.

Preparation of Cell-free filtrate

During the incubation period, 2 mL of the actively growing culture was harvested for four days at a time

Table 1: Experimental design for evaluating antibacterial activity

Sets	DNS-13	DNS-14	DNS-16	Control
Set 1	DNS-13	DNS-14	DNS-16	Only NB
Set 2	DNS-13 + 0.1%	DNS-14 + 0.1%	DNS-16 + 0.1%	NB+ 0.1%
Set 3	DNS-13 + 0.5%	DNS-14 + 0.5%	DNS-16 + 0.5%	NB+ 0.5%
Set 4	DNS-13 + 1.0%	DNS-14 + 1.0%	DNS-16 + 1.0%	NB+ 1.0%

interval of 12 h (12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h, and 96 h). The collected sample was centrifuged at 15,000 rpm for 15 min at -4°C. Following centrifugation, the supernatant was carefully collected and passed through a sterilized membrane filter (0.22 µm pore size) to ensure the sterilization process. The filtered supernatant was transferred to a new sterilized Eppendorf tube, which was further used for the analysis of antimicrobial activity (Cadirci and Citak, 2005).

Agar well diffusion assay

The antibacterial assay was performed by using the agar well diffusion assay (CLSI 2011) with slight modifications. Bacterial pathogens (*S. aureus* and *E. coli*) were grown overnight in nutrient broth in a B.O.D. incubator shaker at 28°C± 2°C. The concentration of the bacterial suspension was adjusted to 2-5 × 10^5 CFU/ml by adding sterile distilled water (Cao *et al*., 2004). One ml of this diluted pathogenic bacterial suspension was seeded into molten nutrient agar medium and poured into Petri plates before solidification. After solidification of the nutrient agar plates, wells were made with a sterilized cork borer, and 50 µl of the supernatant or cell-free filtrate was added into the wells. The plates were incubated in an upright position in an incubator at 28°C± 2°C for 24 h. After the incubation, antibacterial activity was evaluated by measuring the diameter zone of inhibition in millimeters (Reshma and Brindha Priyadarisini, 2021; Manjulatha *et al*., 2024).

Bacterial Growth Kinetics

To determine the appropriate growth phase for bioactive metabolite production, growth kinetics of the selected bacterial isolates (DNS-13, DNS-14 and DNS-15) were evaluated by a growth curve analyzer instrument (Clover Scientific Pvt. Ltd.), which automatically monitors and records the growth of bacteria over time in a 96-well microtiter plate. As instructed in the manual, 300 µl of sterilized uninoculated nutrient broth was used as a control in starting wells. Twenty (20) µl of overnight-grown active bacterial culture was added to wells already supplemented with 280 µl of sterile nutrient broth. Each treatment was kept in triplicate. The plates were incubated and monitored for absorbance at 600 nm every 30 min.

RESULTS AND DISCUSSION

Antimicrobial-resistant pathogens are increasing globally in the health care sector, veterinary medicine, livestock farming, and agricultural practices (Nunez- Serrano *et al*., 2026). This problem has opened a path in the field of research to search for a new, natural and novel source of antimicrobial agents. Considering this, the present study evaluated the antibacterial activity of neem endophytic bacteria DNS-13, DNS-14, and DNS-16 against two clinically relevant bacterial pathogens, Gram-positive *S. aureus* and Gram-negative *E. coli*. Similarly, the effect of the addition of neem leaf powder on the antibacterial property of endophytic bacteria was also tested. The aseptic property of dried neem leaf powder was confirmed, as no microbial growth was observed in the nutrient broth supplemented with sterilized neem powder after seven days of observation at room temperature.

Antibacterial activity of endophytic bacteria

The antibacterial activity of three strains (DNS-13, DNS-14, and DNS-16) and their treatments supplemented with different concentrations of neem powder was checked against *S. aureus* and *E. coli* at a time interval from 12 to 96 h. The inhibition zone (in mm) indicates the bacterial metabolite production during different growth phases, indicating the time-dependent production of antimicrobial metabolites as shown in Table 2.

In the case of *S. aureus,* strain DNS-13 showed a maximum inhibition zone of 16 mm at 36 h, and a 15 mm inhibition zone at 24 h, as shown in Fig 1 (a). When DNS-13 was supplemented with 0.1 % (w/v) neem leaf powder, the maximum inhibition zone was observed at 12 mm at 36 h and 48 h. After 48 h, no prominent inhibition zone was observed. However, when supplemented with 0.5 % neem powder, the antimicrobial activity was similar to 0.1% supplementation. When supplemented with 1 % (w/v) neem powder, delayed activity was observed. Inhibition zone observed in between 48 h

and 72 h, with a maximum zone of inhibition observed at 60 h, which is 15 mm. Overall, these observations represent that the most active phase for metabolite production was from 12 h to 36 h.

In the case of *E. coli*, isolate DNS-13 exhibited a maximum zone of inhibition at the early incubation stage, which is 15 mm at 12 h, and no further inhibition zone was observed for the subsequent incubation period. When supplemented with neem extract, the duration of antimicrobial activity was extended, but the magnitude was less as compared to untreated DNS-13. When supplemented with 0.1 % (w/v) neem powder, an inhibition zone was observed at 12 and 24 h, which was 12 mm for both. Similarly, when supplemented with 0.5% and 1% neem powder, an inhibition zone was observed at 36 h, which was 12 mm. These patterns of observations resulted in a prolonged antibacterial effect as compared to that of untreated DNS-13.

Bacterium DNS-14 showed the highest and most consistent antimicrobial activity among all the isolates. For *Staphylococcus aureus*, untreated DNS-14 showed a maximum inhibition zone of 18 mm at 24 (Fig 1a) and 36 h of incubation. However, further decline and inconsistency were observed in antimicrobial activity up to 10 mm for the remaining incubation period. When the culture was supplemented with neem powder, all the treatments showed antimicrobial activity up to 96 h with stability in the results. In treatments containing 0.1 % and 0.5 % neem powder, inhibition zones of 15 mm and 14 mm, respectively, were observed during the early phase at 12 h. In contrast, DNS-14 metabolite production was delayed in 1 % treated samples, and a zone of inhibition of 20 mm was observed from 24 h. The maximum zone of inhibition against *S. aureus* was observed at 24 h, with a diameter of 18 mm for the sample treated with 0.1 % neem powder. In contrast, 0.5% and 1 % treated samples showed maximum zones of inhibition of 20 mm and 21 mm, respectively, at 36 h. A prominent zone of inhibition was observed at 48 h for DNS-14 supplemented with 1% neem powder, as shown in Figure 1(b). However, the consistency in antibacterial activity throughout the incubation period (up to 96 h) was observed for the culture and remaining treatments. Results also showed that supplementation of neem powder enhances the antimicrobial activity as compared to the non-supplemented.

Against *E. coli*, DNS-14 and its neem-treated cultures showed less antimicrobial activity as compared to *S. aureus*. The untreated DNS-14 culture showed a maximum zone of inhibition of 13 mm at 48 h of incubation, and no inhibition zone was observed during the rest of the incubation period. No inhibition zone was observed for the isolate supplemented with 0.1 % neem powder. When observed for isolates treated with 0.5 and 1 % neem powder, the maximum zone of inhibition was observed at 36 h, which corresponds to 13 mm and 15 mm, respectively. For 0.5 % and 1 %, the treated culture showed a less prominent zone of inhibition for the remaining period. Overall, the antibacterial effect against *E. coli* was relatively weaker compared to *S. aureus*. The antibacterial activity of DNS-14 against both test pathogens showed that the maximum zone of inhibition was observed at 36 h when the culture was supplemented with 1 % neem powder.

Isolate DNS-16 exhibited lower antimicrobial activity as compared to the remaining endophytic bacterial isolates evaluated in the study, as shown in Fig. 1 (a). In the untreated, DNS-16 showed a maximum zone of inhibition at 24 h, measuring 9 mm against *S. aureus.* Thereafter, no prominent inhibition zone was observed. When supplemented with 0.1 % neem powder, a 9 mm inhibition zone from 48 h to 96 h, which is more consistent and for a longer period as compared to the control. For 0.5 % neem powder-supplemented bacterial isolates showed the highest inhibition zone of 12 mm was observed at 24 h, then declined to 10 mm from 48 h to 60 h. In the case of 1 % (w/v) neem powder showed maximum inhibition activity of 11 mm at 36 h. Following this period, 10 mm antimicrobial activity was observed for the remainder of the incubation period. In the case of *E. coli*, DNS-16 does not show any inhibitory effect. When supplemented with 0.1 % and 0.5 % neem powder,

a 11 mm inhibition zone was observed at 24 h. However, when supplemented with 1 % inhibition was observed at 48 h, with a size of 10 mm (Figure 1b). In the case of *E. coli*, *the* DNS-16 isolate does not show any inhibitory effect. When supplemented with 0.1 % and 0.5 % neem powder 11 mm inhibition zone was observed at 24 h. However, when supplemented with 1 % inhibition zone was observed at 48 h of 10 mm in size.

Our findings suggested that all three endophytic cultures exhibit a higher inhibition pattern against *S. aureus* than *E. coli.* This difference in inhibition is due to variation in the cell wall characteristics. The cell wall of Gram-negative bacteria is more complex because it contains an additional outer layer of lipopolysaccharide, which may restrict the entry of antimicrobial agents (Lopez-Romero *et al*., 2015). Out of the three endophytic cultures, maximum antibacterial activity was observed for DNS-14 followed by DNS-13, and DNS-16 in descending order. Metabolite production started in the early phase of growth for DNS-13 and DNS-14 at 12 h. In contrast a delayed activity was observed in DNS-16 at 24 h. When neem leaf powder was supplemented at different concentrations, a delay in antibacterial activity was observed, which may be due to initial metabolic adaptation of the strains. Once established, the magnitude of inhibition was higher than that of the untreated culture. These observations suggest that neem leaf powder acts as an additive for the biosynthesis of antimicrobial metabolites.

In line with our findings, Duong *et al*. (2020) has isolated seven endophytic bacteria from *Azadirachta indica.* Out of all seven, the KT2 strain exhibits antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus* having a zone of inhibition of approx. 11 mm and 10 mm, respectively. This KT2 strain was identified as *Bacillus subtilis* with 87% similarity index. Singh *et al*. (2017) isolated 25 endophytic bacteria from neem leaf, and antimicrobial activity was assessed against various pathogenic Gram-positive and Gram-negative bacteria by disk diffusion assay. The results indicate that 80% of these isolates inhibit the *S. aureus*, 84% inhibit *Streptococcus pyogenes*, 8% inhibit *Bacillus cereus.* Similarly, they also inhibit the Gram-negative bacteria 92% of them inhibit the *E. coli*, 88% showed inhibitory activity against *Salmonella typhimurium* and *Klebsiella pneumoniae*. These findings reinforce the broad-spectrum antimicrobial potential of neem-associated endophytic bacteria.

In contrast to the current study, which focused on endophytic bacteria, Nthuku *et al*. (2023) isolated endophytic fungi from neem, and their antagonistic activity was assessed against *Fusarium oxysporum*

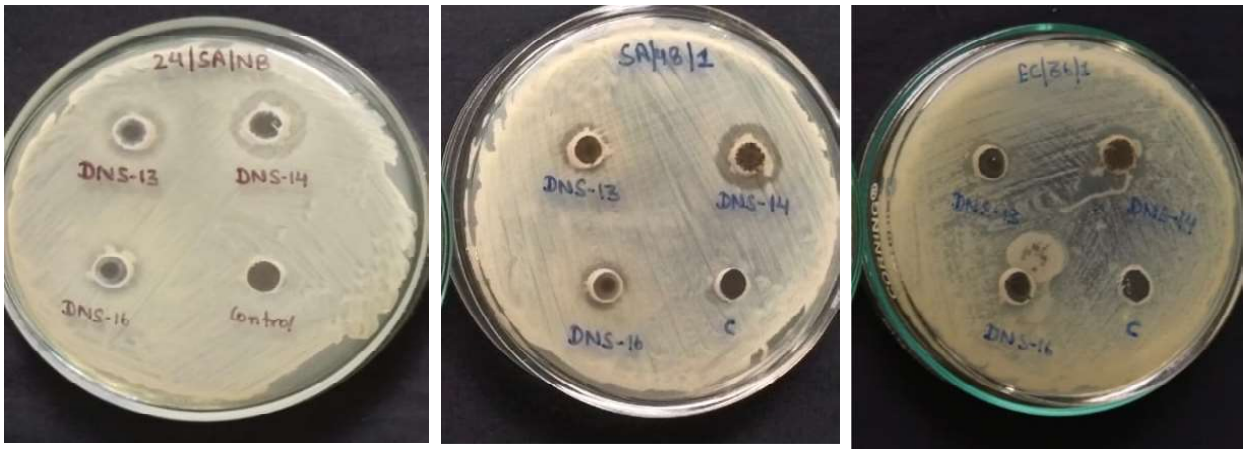

Fig. 1: Representative plates showing the antimicrobial activity of selected bacterial isolates against *Staphylococcus aureus* and *Escherichia coli* under different culture conditions. (a) Antagonistic activity of 24 h-old bacterial cultures against *S. aureus*. (b) Antagonistic activity of bacterial isolates after 48 h of incubation in medium supplemented with 1% neem against *S. aureus*. (c) Antagonistic activity of bacterial isolates after 36 h of incubation in medium supplemented with 1% neem against *E. coli*.

f. sp. *cubense* TR4 which causes Panama disease in banana by dual plate assay. It was found that *Alternaria alternata* NR3 and *Alternaria tenuissima* NB6 show percent inhibition by 93.94 % and 84.82%, respectively, against *Fusarium oxysporum*. One of the recent studies reported that 30 endophytic bacteria were isolated from different tissues of the neem plant and checked for antagonistic activity against *Xanthomonas campestris* pv. *vesicatoria*. Among these, *Bacillus safensis* LE8 and *Pseudomonas lactis* LE11 showed percent inhibition of 50.32 and 41.12 %, respectively (Fernandes *et al*., 2026). These findings suggested that endophytic isolates are also effective as biocontrol agents against phytopathogens.

To the best of our knowledge, this is the first study in which an attempt was made to evaluate the influence of neem leaf powder on the antibacterial potential of the same plant-derived endophytic

Table 2: Influence of different concentrations of neem powder on the antibacterial activity of neem-derived endophytic bacterial isolates DNS-13, DNS-14, and DNS-16 against *S. aureus* over different incubation periods

Treatments	12h	24h	36h	48h	60h	72h	84h	96h
NB	0	0	0	0	0	0	0	0
NB+0.1%	0	0	0	0	0	0	0	0
NB+0.5%	0	0	0	0	0	0	0	0
NB+1.0%	0	0	0	0	0	0	0	0
DNS-13	11	15	16	10	0	0	0	0
DNS-13+0.1%	0	0	12	12	0	0	0	0
DNS-13+0.5%	0	0	12	12	0	0	0	0
DNS-13+1.0%	0	0	0	10	15	12	0	0
DNS-14	15	18	18	10	10	10	10	10
DNS-14+0.1%	15	18	15	13	10	10	15	15
DNS-14+0.5%	14	18	20	15	15	15	15	15
DNS-14+1.0%	0	20	21	18	16	15	15	15
DNS-16	0	9	0	0	0	0	0	0
DNS-16+0.1 %	0	0	0	9	9	9	9	9
DNS-16+0.5%	0	12	9	10	10	10	10	10
DNS-16+1.0%	0	0	11	10	10	10	10	10

Values represent the diameter of the inhibition zone (mm), '0' indicates no inhibition zone, NB is Nutrient Broth

Table 3: Influence of different concentrations of neem powder on the antibacterial activity of neem-derived endophytic bacterial isolates DNS-13, DNS-14, and DNS-16 against *E. coli* over different incubation periods

Treatments	12h	24h	36h	48h	60h	72h	84h	96h
NB	0	0	0	0	0	0	0	0
NB+0.1%	0	0	0	0	0	0	0	0
NB+0.5%	0	0	0	0	0	0	0	0
NB+1.0%	0	0	0	0	0	0	0	0
DNS-13	15	0	0	0	0	0	0	0
DNS-13+0.1%	12	12	0	0	0	0	0	0
DNS-13+0.5%	0	0	12	0	0	0	0	0
DNS-13+1.0%	0	0	12	0	0	0	0	0
DNS-14	0	0	0	13	0	0	0	0
DNS-14+0.1%	0	0	0	0	0	0	0	0
DNS-14+0.5%	0	0	13	10	0	0	0	0
DNS-14+1%	0	0	15	13	0	0	0	0
DNS-16	0	0	0	0	0	0	0	0
DNS-16+0.1%	0	11	0	0	0	0	0	0
DNS-16+0.5%	0	11	0	0	0	0	0	0
DNS-16+1.0%	0	0	0	10	0	0	0	0

Values represent the diameter of the inhibition zone (mm), '0' indicates no inhibition zone, NB is Nutrient Broth.

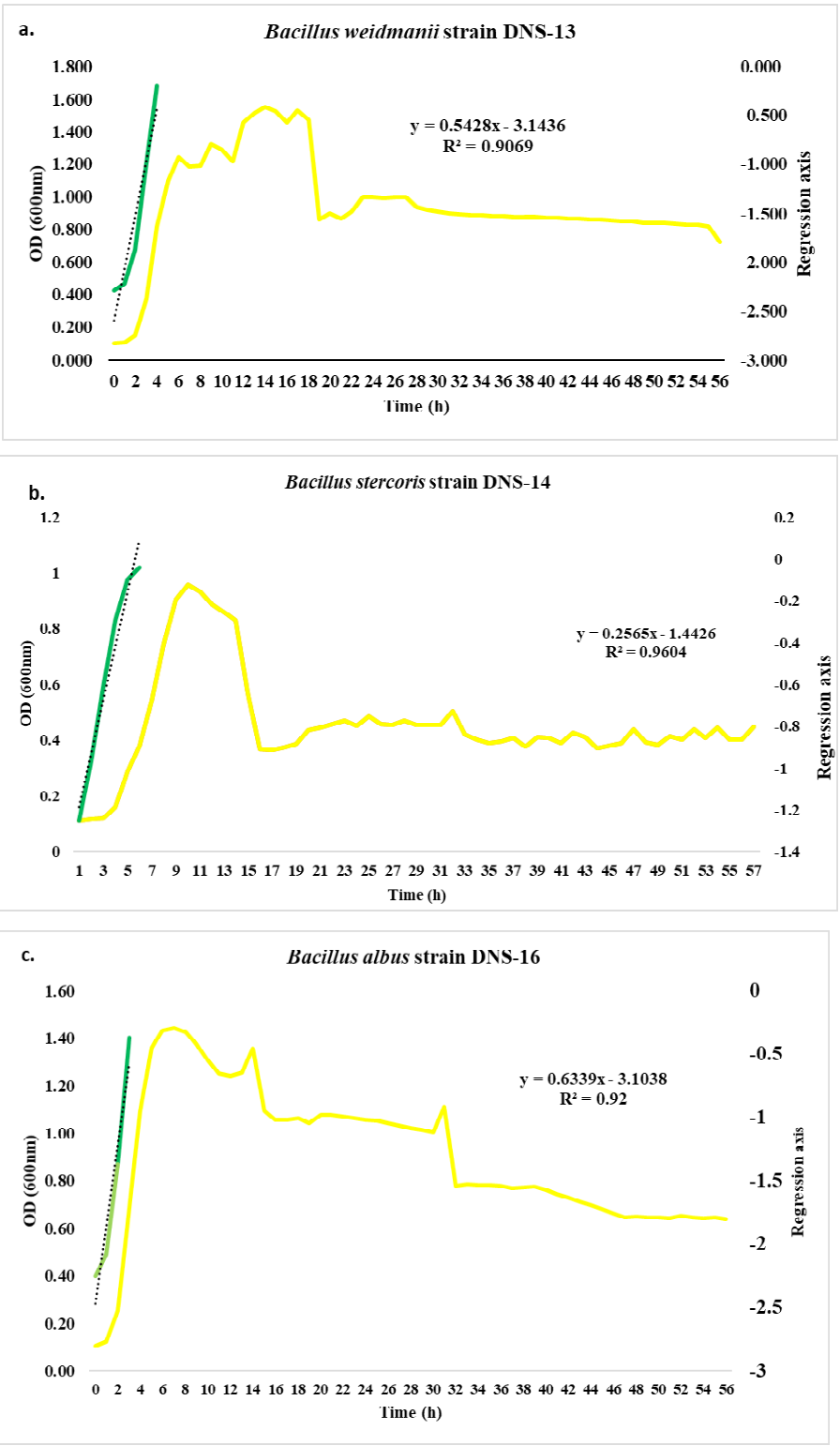

Fig. 3: Growth kinetics and Regression analysis of the stem endophytic isolates (a) *Bacillus weidmanii* DNS-13; (b) *Bacillus stercoris* DNS-14; (c) *Bacillus albus* DNS-16 (where, yellow line indicates absorbance, green line indicates exponential growth phase, and black dotted line represents linear regression)

bacteria. In one of the previous studies, *Azadirachta indica* oil cake has been used in semi-solid state fermentation for efficient and large-scale production of industrially important enzymes, lipase from *Pseudomonas aeruginosa* JCM5962(T) (Sachan and Singh, 2017). Our findings are consistent with the study demonstrating that co-substrate supplementation enhances metabolite production. Submerged fermentation of *Penicillium crustosum* OR889307, when supplemented with lemon peel as a co-substrate, increased the production of bioactive compounds. The semi-purified extract of fermentation shows MIC values of 185 µg/mL against *Pseudomonas aeruginosa* and 225 µg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA). These results support that agro-waste substrate helps in improving the bioactivity of the biocontrol agents (Nunez-Serrano *et al*., 2026).

Growth curve

Antimicrobial activity of bacteria is a growth phase-dependent property. The growth kinetics graph depicted in Figure 2 shows the typical bacterial growth pattern with lag, exponential, and stationary phases. The growth kinetics of the endophytic isolates were studied to demonstrate the bacterial growth pattern by monitoring the optical density over time (Krishnamurthi *et al*., 2021). During the exponential phase, rapid cell proliferation occurs, which leads to biomass accumulation. As the culture enters into the stationary phase multiplication of cells slow down, and leads to the accumulation of secondary metabolites. Prolonged incubation leads to the accumulation of inhibitory by-products, which may alter the antibacterial activity of the isolates (Ochi and Ohsawa, 1984; Chodkowski and Shade, 2020).

Therefore, the growth kinetics of the selected endophytic bacterial isolates were studied to identify the phase suitable for secondary metabolite production and to establish the relationship between secondary metabolite production phase and antibacterial activity.

The growth curve of isolate DNS-13 showed a small lag phase, 0 to 2 h, followed by an exponential phase from 2-6 h, during which the geometric progression of cell growth was observed. The stationary phase started after 6 h, as shown in Fig. 2 (a). The growth rate of DNS-13 was found to be 0.54 h^{-1}. The growth showed that secondary metabolite production was highest during the stationary phase from 12-36 h, which strongly supports the antimicrobial activity results as shown in Table 2. After 36 h, metabolic activity became constant due to the accumulation of metabolic by-products and nutrient depletion. This might be the reason for consistency in the zone of inhibition even after 36 h. A similar observation has been reported by Hasan *et al*. (2009) that the cell-free extract of *Bacillus pumilus* SAF1 shows antagonistic activity against *Micrococcus luteus* and *Staphylococcus aureus* and antimicrobial compound production was initiated at the end of the exponential phase and was maximum at the stationary phase.

The growth curve of DNS-14 showed a lag phase up to 3 h, and then there was a sharp increase in optical density from 4 to 10 h, which represents the exponential phase where the cells multiply rapidly. The growth rate of DNS-14 was found to be 0.26 h^{-1} (Fig. 2 (b)). A prolonged stationary phase was observed after 10 h. The prolonged stationary phase is often associated with secondary metabolite production, which explains the consistent antimicrobial activity as observed in Table 2.

In similar way, the growth curve of DNS-16 showed a lag phase up to 2 h, and then there is a sharp increase in optical density from 3 to 6 h, which represents the exponential phase where the cells multiply rapidly. The stationary phase started after 6 h and the growth rate was found to be 0.64 h^{-1} as shown in Fig. 2 (c). Thus, the results showed a correlation between the bacterial growth and antibacterial activity. Thus, understanding the growth pattern is essential to know the specific time of harvest of cells for extraction of antibacterial metabolites.

CONCLUSION

The present study findings demonstrate that endophytic bacteria *Bacillus weidmanii* DNS-13, *B.*

stercoris DNS-14 and *B. albus* DNS-16 exhibit antibacterial activity against *S. aureus* and *E. coli*. All three endophytic strains exhibit greater antibacterial activity against *S. aureus* than *E. coli*. Endophytic bacterial isolate DNS-14 showed the maximum inhibition, followed by DNS-13 and DNS-16. The growth kinetics and antibacterial assay at different incubation periods revealed that the antibacterial activity was higher in the stationary period of 24 to 36 h. The supplementation of neem leaf powder increased the antibacterial activity compared to the control (non-supplemented). Collectively, all the findings suggest that neem endophytic bacteria are a promising candidate for the development of an antimicrobial agent to combat with antimicrobial resistance.

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Received: June 20, 2026
Accepted: August 04, 2026