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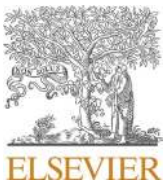
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Identification and characterization of the pathogens associated with tea red rust in India and their biocontrol under field conditions

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HIGHLIGHTS

- *Cephaleuros virescens* and *C. parasiticus* from tea red rust were identified.
- Stable 7-month liquid formulations from *B. paramycoides* and *M. barkeri* were made.
- Both formulations effectively controlled tea red rust in lab and field tests.
- *B. paramycoides* and *M. barkeri* are promising agents for tea red rust control.

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ABSTRACT

Red rust of tea, caused by *Cephaleuros* spp., is an algal disease that can lead to substantial yield losses in major tea-growing countries, including India. This study first identifies and characterizes the causal agents associated with algal red rust disease and then focuses on their field management through a 3-year field trial (2021–2023) across three experimental sites. Micro-morphological, host invasion, and phylogenetic (18S SSU) analysis revealed the association of *Cephaleuros virescens* (subcuticular) and *C. parasiticus* (intercellular) with the disease. Liquid bioformulations of two bacterial antagonists, namely *Bacillus paramycoides* BOK01 and *Microbacterium barkeri* BPATH02, were prepared and tested against the pathogens under laboratory and field conditions. The efficacy of the biocontrol formulations was compared with a copper-based chemical (i.e., copper oxychloride), commonly used in India. Under controlled conditions, a 20 % suspension concentrate liquid formulation (1×10^8 CFU/ml) of both bacterial antagonists reduced over 66 % spore viability of *Cephaleuros* species at 2 % concentration. Field trials of the two bacterial-based bioformulations showed over 60 % disease reduction at 2 % and 5 % concentrations compared to the control. Both bioformulations were viable for up to seven months of storage, exhibiting a high population density (i.e., $>1 \times 10^8$ CFU/ml). Copper oxychloride provided significantly higher disease reduction (>68 %) than the bacterial bioformulations across all locations and years, but no significant yield difference occurred between the two treatments. Taken together, we conclude that *B. paramycoides* BOK01 and *M. barkeri* BPATH02-based liquid bioformulations have good potential in the integrated management of algal red rust in tea gardens.

1. Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is mainly grown in Asia and Africa on over 26.61 million ha, with a total annual production of 29.8 million tons (Mjanja et al., 2025). China is the major tea producer, followed by India. The Indian tea industry contributes 28 % of the global tea production, with approximately 1.382 million tons of tea per year from 3.69 million ha of cultivated areas (Mjanja et al., 2025). Among

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many biotic and abiotic factors that affect tea production in India, algal red rust (ARR) is an economically important disease in the country.

While ARR is a minor problem in China and Sri Lanka (Sinniah, 2018; Fang et al., 2021), the disease is the most prevalent in India and Bangladesh (Barthakur, 2011; Pandey et al., 2023a; Islam and Himel, 2023), causing 40–60 % annual yield losses (Ramya et al., 2013). The perennial monoculture, poor agronomic practices (i.e., poor drainage, dense shade, alkaline pH), and warm and humid climates trigger ARR epidemics. Both pathogens attack the stem and leaves, and they can only be differentiated based on the penetration inside the cortex. *Cephaleuros parasiticus* can penetrate the epidermis, while *C. virescens* cannot. Severe disease development results in reduced photosynthesis and causes significant defoliation of tea plants (Barthakur, 2011; Huq et al., 2022). ARR attacks young and mature tea plants by damaging maintenance foliage in patches. The algae produce minute zoospores within sporangia, especially on the stems as well as on the lower surface of the leaves. Sporangia are detached after maturation and easily spread to host plants (*Albizzia* species and Bogamedeloa), where spores are released (Huq et al., 2010).

ARR has become a significant problem during the last two decades in Indian tea gardens due to the widespread use of machines for harvesting tea shoots, and the disease is further triggered by climate change (Gnanamangai et al., 2011). Besides causing yield losses, ARR also affects physiological and biochemical parameters, thereby deteriorating tea quality (Devarajan et al., 2018; Ponmurugan et al., 2007). Several factors predispose to disease, including poor soil fertility, inadequate shade, insufficient soil aeration, extreme drought, improper fertilizer application, frost, waterlogging, hard plucking, and continuous shear harvesting (Pandey et al., 2023b). Infected plants become weak and their new shoots are thin with heavy banji shoots, affecting the tea quality (Bore, 1996; Islam and Himel, 2023; Pandey et al., 2023b). The pathogen reproduces by both sexual and asexual means; however asexual stage plays a crucial role in the disease cycle (Vasconcelos et al., 2018). The pathogen survives year-round on existing leaves and stems as spores and in crop debris. The mode of disease transmission through wind and water makes disease management difficult in tea gardens.

Algal red rust is caused by two obligate parasitic chlorophyte algal species worldwide, namely, *Cephaleuros parasiticus* Karst. and *Cephaleuros virescens* (Johnson and Hobman, 1982; Ponmurugan et al., 2010; Barthakur, 2011). The pathogen, *Cephaleuros* spp. (Ulvophyceae: Trentepohliales), is a chlorophytic alga that infects over 250 plant species (Nelson, 2008), particularly tree crops. In India, there is no consensus regarding the pathogens associated with ARR on tea, with one study revealing *C. parasiticus* as the unique causal agent (Ramya et al., 2013) and another one reporting *C. parasiticus* and *C. virescens* as the causal agents (Barthakur, 2011). This calls for an accurate identification and characterization of the pathogens causing ARR, which is essential for developing and deploying appropriate disease management strategies. Several studies used morphological and molecular methods to identify and differentiate *Cephaleuros* species from various crops, including mango (Vasconcelos et al., 2019, Vasconcelos et al., 2018) and other fruit crops (Huang et al., 2023). Significant effort has been made on the molecular identification of *C. parasiticus* (Vasconcelos et al., 2019) and *C. virescens* (Patrice et al., 2021) using various techniques, including 18S small subunit (Lopez-Bautista et al., 2006) and ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rbcL*) gene (Fang et al., 2021; Rindi et al., 2009). In India, ARR pathogens were characterized based on host invasion and morphological features (Ponmurugan et al., 2010) without providing any molecular evidence.

Tea growers in India adopt integrated disease management combining cultural, chemical, and biological approaches. Cultural management includes the correction of soil pH, improvement of drainage, shade management, and application of fertilizers like muriate of potash (Barthakur, 2011). Synthetic agrochemicals like copper oxychloride and copper hydroxide are commonly used in India (Barthakur, 2011; Ramya et al., 2013) and Bangladesh (Huq et al., 2010; Islam and Himel, 2023). In contrast, copper hydroxide is not approved in India against tea ARR (Plant Protection Code, 2023), although it has been used for the management of *C. virescens* infecting litchi (Garima et al., 2024), *C. parasiticus* in mango (Kumar et al., 2022), and guava (Kalita and Srivastava, 2022). A frequent use of copper oxychloride is neither cost-effective nor environmentally friendly, discouraging its use in tea gardens for not to mention the risks of resistance development by *Cephaleuros* species. Microbial biocontrol agents represent valid alternatives to copper compounds in managing ARR in India as they are cost-effective, eco- and farmer-friendly, particularly in organic farming.

Among microbial biocontrol agents, *Streptomyces sannanensis* and *S. griseus* have shown efficacy against tea ARR in India (Ramya et al., 2013), while *Bacillus, Pseudomonas, Streptomyces,* and *Trichoderma* species were effective against *C. parasiticus* in Bangladesh (Huq et al., 2022). Recently, *Trichoderma reesei* and actinobacterial species have been successfully applied to manage tea Fusarium dieback (Babu et al., 2022; Pandey et al., 2023c) and gray blight in India (Pandey et al., 2022). Several bacterial and fungal secondary metabolites are effective against many plant pathogens (Hamdali et al., 2008; Rutledge and Challis, 2015). However, no bioproducts are available for ARR management in Indian tea gardens. Therefore, the objectives of the present study were to i) identify the pathogens associated with ARR on tea, ii) characterize bacterial antagonists based on 16S rRNA sequencing and scanning electron microscopy, and iii) evaluate bacterial antagonist-based liquid formulations for their functional and ARR-controlling properties under laboratory and field conditions.

2. Materials and methods

2.1. Study of the algal red rust pathogen

2.1.1. Collection of infected plant samples and host invasion analysis

During 2020–2023, leaf samples of 10–20 years old tea cultivar Teenali showing algal spots, both epiphytic and parasitic stages, were collected from Sannyasithan (132 m asl; 26.6 N° 88.2 °E), Jiti (320 m asl; 26.9 °N 88.9 °E), and North Bengal Regional Research and Development Centre, Nagrakata (NBRRDC; 214 m asl; 26.8 °N 88.9 °E) tea gardens of North Bengal and Sessa (58 m asl; 26.7 °N 92.7 °E) and Hatikhuli (81 m asl; 26.5 °N 93.1 °E) tea gardens of Assam, India. Three replicates, each consisting of a set of 15 leaves, were sampled from each location. The samples were brought to the Mycology Laboratory of TRA, NBRRDC, Nagrakata, to isolate pathogens. In total, one epiphytic and parasitic leaf samples were collected from each tea garden in a Zip log bag with assigned codes SNYR01, SNYR02 for Sannyasithan, JITR01, JITR02 for Jiti, NKR01, NKR02 for NBRRDC, Nagrakata, SSR01, SSR02 for Sessa, and HTKR01, HTKR02 for Hatikhuli tea gardens. To determine the type of host invasion and phylogenetic analysis from the same isolate, one-half samples were used for the study of the type of invasion, and the other half for the isolation of algal pathogens and DNA extraction. The host invasion type was studied using the methodology of Huang et al. (2023). The algal isolates were identified as either intercellular or subcuticular type based on the absence or presence of algal filaments/sporangia in the cortical region (Huang et al., 2023).

2.1.2. Pathogen isolation and cultural characterization

The infected leaves from each replicate were chopped into small pieces across the algal spots and sterilized using 1 % sodium hypochlorite. The small infected fragment of each sample was aseptically transferred into a conical flask (250 ml) containing 100 ml of Potato dextrose, Czapek-dox, and Trebouxia broths supplemented with streptomycin for isolation of the pathogen. The inoculated flasks were kept in the Biochemical Oxygen Demand incubator (Rivotek, Mumbai, India) at 25 °C and 12 h white light for 5 days (Vasconcelos et al., 2018). The algal mycelium obtained through liquid broth was transferred to Petri dishes containing 10 ml of Potato dextrose, Czapek-dox, and Trebouxia agar media supplemented with streptomycin (100 mg). The growth of each

isolate was examined 7 days post-incubation, and the diameter of colonies grown on agar surface was measured in three replicates.

2.1.3. DNA extraction and polymerase chain reaction

The genomic DNA (gDNA) from algal spots from symptomatic leaves of each isolate was extracted using a DNA extraction kit (QIAGEN, Germany) following the manufacturer's protocols. The integrity and quantity of DNA were checked with 0.8 % gel electrophoresis and Nanodrop (Thermo Fisher Scientific, India) at 260/280 nm of absorbance, respectively. The polymerase chain reaction (PCR) amplification of the 18S Small Subunit of each isolate was conducted using universal primers 18SHf and 18SLr (Hamby et al., 1988) in a thermocycler (Bio-Rad, Mumbai, India). The total volume of the reaction mixture was 50 µl, which consisted of 60 ng DNA, Taq DNA polymerase (1X), $MgCl_2$ (3.7 mM), dNTPs (0.6 pmol/µl), primer (each 0.4 pmol/µl), and Taq DNA polymerase (5U). The PCR conditions were 95 °C for 3 min, 30 cycles for 1 min each at 95 °C and 52 °C, 2 min at 72 °C, and final extension at 72 °C for 10 min. The amplified PCR products of each isolate were visualized in a 1 % agarose gel and observed under UV light after staining with EtBr. The amplicons were further purified using a UniPro Gel extraction kit (Macrogen, Inc., Korea) for sequencing. The sequencing was performed at Barcode Bioscience, Bangalore, Karnataka, India. The sequence editing and assembling were conducted in BioEdit, and the identity of each isolate was confirmed through the NCBI GenBank database, using the BLAST analysis.

2.1.4. Phylogenetic analysis

Phylogenetic association among the present ARR isolates was based on the Small Subunit marker and analyzed in MEGA X using the maximum likelihood method (Kumar et al., 2016). The reference sequences of both *Cephaleuros* species were retrieved from NCBI GenBank, and their phylogenetic relationship with our isolates was determined in MEGA X. Before the phylogenetic analysis, the nucleotide substitution model was determined using the function "Find Best DNA/Protein Models" (Huang et al., 2023). In each phylogenetic analysis, 1000 bootstrap replicates were employed. Bayesian inference analysis was conducted using MrBayes V. 3.2.7, adopting the Ronquist and Huelsenbeck (2003). An analysis of Markov chain Monte Carlo was performed for one million generations with sampling trees every 100 generations. Split frequencies with standard deviations less than 0.05 were expected to show a stationary distribution. As burn-in, the first 25 % of the trees were rejected, and the outcomes were expressed as posterior probabilities. The sequence of *Trentepohlia aurea* was used as an outgroup.

2.2. Rhizobacterial antagonists

2.2.1. Isolation and morpho-cultural characterization

The bacterial antagonists, namely *Bacillus paramycoides* and *Microbacterium barkeri* with assigned codes BOK01 and BPATH02, evaluated against ARR in this study, were isolated from soil samples collected from Okyati (1820 m asl; 26.6 °N 88.9 °E) and Patharjhora (141 m asl; 26.9 °N 88.6 °E) organic tea gardens in 2020, North Bengal, respectively, through the serial dilution technique on the nutrient agar plate. These isolates were found to be effective against pathogens causing tea gray blight, Fusarium dieback, and blister blight, produced antifungal metabolites, and have potential plant growth promotion properties (Pandey et al., 2023b). The colony characteristics of each bacterial isolate were examined by culturing them onto Luria Bertani agar plates for 48 h at 32 °C in an incubator and then observed under the light microscope (Olympus, Mumbai, India) after Gram staining for their size and shape. For the scanning electron microscope study, the cultures of each isolate were washed with isoamyl acetate (each 3 min) at 50, 70, 90, and 100 % concentrations, dried aseptically, followed by coating with palladium. The mounted samples were analyzed under Scanning Electron Microscopy (JEOL JSM-IT100) at North Bengal University and photographed in three replicates following the methodology of Wang et al. (2020). Besides, the catalase test for both bacterial antagonists was also performed (Bhattacharyya et al., 2020).

2.2.2. Molecular characterization and phylogenetic analysis

The QIAamp DNA Mini kit (Qiagen, India) was used for the extraction of total gDNA from each bacterial isolate. The quality and quantity of extracted gDNA were determined as previously described (Pandey et al., 2023b). The sequencing of the 16S rRNA gene for each isolate was carried out using 27F and 1492R primer pairs, adopting the PCR conditions used by Weisburg et al. (1991). The purified PCR product of each isolate was sequenced at Eurofins Analytical Services, Bangalore, Karnataka, India. The obtained sequences of each isolate were edited and further subjected to BLAST analysis in NCBI GenBank for a similarity test (Altschul et al., 1990). The phylogenetic tree was constructed in MEGA X based on the Kimura 2-parameter model (Tamura and Nei, 1993) following the Maximum Likelihood method (Kumar et al., 2016). The consistency of internal branches was assessed from 1000 bootstrap replications (Felsenstein, 1985). The internal tree was obtained with the help of BioNJ algorithms and Neighbor-Join techniques. The positions of codon were 1st + 2nd + 3rd + Noncoding regions. The phylogenetic tree was constructed using sequences of bacterial species examined in this study, along with four nucleotide sequences of each associated species of each isolate retrieved from GenBank. The sequence of *Pseudomonas aeruginosa* was used as an outgroup.

2.3. Evaluation of bacterial antagonists in laboratory conditions

2.3.1. Preparation of liquid formulation of bacterial antagonists

The inoculum of both bacterial antagonists was prepared on sorghum extract. Each bacterial isolate was inoculated in 1 L of sorghum liquid broth (sorghum powder 10 g/L, sucrose 20 g/L) and incubated at 28 °C for 48–72 h, in three replicates in an incubator. A 20 % suspension concentrate (4 L sorghum-based concentrate broth culture + 16 L distilled water) formulation (v/v) of each bacterial antagonist was prepared in distilled water from liquid broth, and 0.25 % yeast extract, 1 % sugar, 0.2 % glycerine, and 1 % carboxyl methyl cellulose were mixed aseptically on a laminar air flow. The inoculum density of developed formulations was counted through the serial dilution technique on nutrient agar plates. The stock liquid formulation (20 % Suspension Concentrate) with a population of 1×10^8 CFU/ml of each bacterial antagonist was used for lab and field experiments. Concentrations of 1 %, 2 %, and 5 % (v/v) of each bacterial antagonist-based formulation prepared in distilled water were separately used for bio-efficacy tests against ARR.

2.3.2. Efficacy of bacterial antagonists-based formulations in the laboratory

In 2021, leaves showing fresh ARR spots/discs (mixture of both *C. virescens* and *C. parasiticus*) were collected from the Teenali tea cultivar from the research plot of TRA, NBRRDC, Nagrakata, West Bengal, and brought to the Mycology laboratory for efficacy tests. The leaves were kept in pre-sterilized Petri plates containing three layers of blotter paper. The petiole of each leaf was wrapped with cotton soaked with double-distilled water to prevent the leaf from drying, and the cotton was sprinkled with water at 2-day intervals to maintain moisture. Concentrations of 1 %, 2 %, and 5 % of each formulation and copper oxychloride (2.5 g/L) were sprayed on algal discs twice at 3-day intervals using a glass automizer. The leaves without spray were served as control sets. All the experimental plates were incubated in three replicates in a complete randomized design in an incubator at 32 °C with 12 h of white light. After incubation, each algal disc was transferred into Trebouxia liquid media to confirm viability of spores, and results were determined in terms of % reduction in spore viability by bacterial antagonist-based formulations, following the formula:

$$\%\,\text{Reduction} = \frac{N0 - Nd}{N0} \times 100$$

Where N0 was the initial number of algal discs before treatment, and Nd was the number of algal discs that did not grow on Trebouxia liquid medium. In addition, the viability of spores was also verified by a spore germination test under a light microscope. The experiments were repeated once under the same conditions.

2.3.3. Evaluation of bacterial antagonists for their physiological traits

Both bacterial antagonists were evaluated for various physiological properties such as melanin production, starch hydrolysis, salinity test, and growth on different ranges of pH and temperature. The intensity of melanin production was determined qualitatively following the methodology of Ara et al. (2012) using the peptone, iron, and ISP7 agar media. Starch hydrolysis bioassay was carried out by flooding a 7-day-old bacterial culture cultivated in starch agar medium with a 2 % solution of Gram's iodine. The transparent zones formed around the bacterial colonies indicated the starch-hydrolysing attributes of each isolate.

For salinity tolerance determination, peptone broth (100 mL) containing 300, 600, 900, 1200, and 1500-mM concentrations of NaCl was inoculated with isolates BOK1 and BPATH02 separately, and incubated at 28 °C in a BOD incubator. To evaluate the growth of bacterial antagonists at different temperatures and pH ranges, each bacterial antagonist was inoculated in a conical flask (250 mL) containing 100 mL peptone broth. The impact of pH on the growth of bacterial antagonists was determined by altering the pH of culture media in the range of 2–9 and incubating broths at 28 °C. Likewise, the effect of temperature on the growth of each bacterial antagonist was assessed by incubating bacterial-inoculated broths at 5–45 °C temperature ranges. After 48 h of post-incubation, the population density was computed through the serial dilution technique, and values were presented as log 10 CFU/ml (Li and Feng, 2009). Each experiment was repeated once under the same conditions.

2.4. Study of shelf life of bacterial-antagonist-based bioformulations

The shelf life of developed formulations of both bacterial antagonists stored at room temperature (28 ± 2°C) was determined through the serial dilution technique at monthly intervals. Nutrient agar plates spread with stock, 10^{-4} and 10^{-8} dilutions of each bacterial formulation on agar surface were incubated at 32 °C in an incubator in three replicates, and results were presented as CFU/ml. Each experiment was repeated once under the same conditions.

2.5. Evaluation of bacterial antagonists-based formulations under field conditions

2.5.1. Description of the study sites

A field trial was conducted across three experimental sites in three consecutive years (2021–2023). Three tea gardens, namely Jiti, Sannyasithan, and NBRRDC, Nagrakata, West Bengal, India, were selected based on the high ARR development in previous years during rainy seasons (June to September). Each tea garden had 5- to 10-year-old highly susceptible tea cultivar, Teenali, infected with both *C. parasiticus* and *C. virescens* in the rainy season. The standard agronomic and cultural practices like pruning, plucking, manuring, and weeding were adopted in the experimental plots at each location as per Tea Research Association recommendations (Barthakur, 2011). At each location, other pests and diseases were managed following the Field Management Tea guide developed by the Tea Research Association, India.

The weather data for each location was obtained from the respective tea gardens. Daywise data for relative humidity, temperature, and rainfall (precipitation) were collected during the evaluation period for each year. The mean relative humidity, temperature, and cumulative rainfall values were computed (Table 1S). The soil texture of all experimental sites was sandy loam (43–50 % sand, <50 % silt, and < 7 % clay), while soil pH and percent organic carbon status are as follows: Jiti tea garden pH 4.8 ± 0.1 with 1.7 ± 0.4 % organic carbon, NBBRDC, Nagrakata tea garden 4.7 ± 0.2 with 2.0 ± 0.8 % organic carbon, Sannyasithan tea garden 4.8 ± 0.2 with 1.6 ± 2.0 % organic carbon.

2.5.2. Impact of bacterial antagonists-based formulations on algal red rust disease

The efficacy of bacterial antagonists-based bioformulations against ARR (both epiphytic and parasitic stage) infected tea bushes was assessed by spraying the following treatments: T1, T2, T3 were 1 %, 2 %, 5 % *B. paramycoides* BOK01, T4, T5, T6 were 1 %, 2 %, 5 % *M. barkeri* BPATH02, and T7, T8, T9 were 2 % commercial *Bacillus subtilis*, copper oxychloride 50 % WP (2.5 g/L), and control (without spray), respectively. The field trials were conducted in a randomized complete block design with three replicates, each containing 50 tea bushes. The plot size was 37 m^2, with a spacing of 110 cm × 75 cm and optimum shade status. Each treatment was separated by a guard row of untreated tea bushes. Two rounds of each treatment were sprayed on tea bushes in the morning hours at 15-day intervals by a hand-operated knapsack sprayer (discharge 450 ml/min at 40 psi pressure, 400 L/ha). Each bush was properly drenched for well coverage and management of ARR disease.

2.5.3. Phytotoxic effect

To evaluate the phytotoxicity effect (i.e., yellowing, necrosis, stunting, hyponasty, epinasty, etc.) of previously described various concentrations of bacterial antagonists-based formulations (>1 × 10^8 CFU/ml) on tea leaves, experiments were carried out at the research plot of NBRRDC, Nagrakata, West Bengal, in three replicates in 2022 and 2023. The plot size was 37 m^2, and the experiment was conducted in a randomized complete block design. The control set was included without treatment. The results on phytotoxicity on tea leaves were examined at 0 day, 3 days, 7 days, and 14 days after post treatment and injury levels were scored using the 0-to-10 rating scales, where 0 = no crop response/crop injury, 1 = 1–10 %, 2 = 11–20 %, 3 = 21–30 %, 4 = 31–40 %, 5 = 41–50 %, 6 = 51–60 %, 7 = 61–70 %, 8 = 71–80 %, 9 = 81–90 %, 10 = 91–100 % (Babu et al., 2008). To determine the bacterial antagonist survival, leaf samples were collected every month at the NBRRDC experimental location. Enumeration of *Bacillus* and *Microbacterium* species was carried out using the selective medium, namely *Bacillus* differentiation and Kenk agar media, respectively, following the dilution plate technique (Elad and Chet, 1987).

2.5.4. Disease assessment

Disease severity (coverage of bush) data were recorded by examining the typical ARR symptoms. Out of 50 bushes, 30 bushes were selected randomly to examine for disease severity, and each of them was visually evaluated from each plot just before spraying and at 15-day intervals after the first spray up to 30 days. Disease severity score over time was recorded on a 0-to-5 point rating scale, where 0 = no infection, 1 = 0.1–10 % infection (>80 % recovery of bushes), 2 = 10.1–25 % infection (>61 % and < 80 % recovery of bushes), 3 = 25.1–50 % infection (>41 % and < 60 % recovery of bushes), 4 = 50.1–75 % infection (>21 % and < 40 % recovery of bushes), and 5= >75 % (<20 % recovery of bushes). The disease severity was presented in the percent disease reduction over control, which was computed following a standard formula as described below. Percent disease reduction (PDR) = Disease severity score in untreated plots- Disease severity score in treatment plots/ Disease severity score in untreated plots × 100 (Islam and Ali, 1970).

2.6. Crop yield

Green leaf yield at each location was measured during the field trials in each year. Green leaf yield after 15 days of spray was estimated for the

first six rounds of plucking by keeping a seven-day interval between standard plucking rounds. Average yield was expressed in kg/plot. The green leaf yield, documented at every plucking interval, was converted into processed/made tea for one ha as described previously (Anonymous, 2016).

$$\text{Made tea yield (kg/ha)} = \frac{\text{Green leaf yield (kg/plot)} \times 10,000.00\,\text{m}^2}{\text{Net plot area (m}^2\text{)}} \times 0.225$$

2.7. Statistical analysis

Data from the field and lab experiments were analysed using an analysis of variance (ANOVA) in R software. Replications and treatments were considered as random and fixed effects, respectively. In this study, homogeneity of variance was tested using Levene's test on repeat laboratory experiments that showed no significant differences; hence, the results were combined in the analysis. To test for deviations from normality, Shapiro-Wilk tests were conducted on the standardized residuals from the model, which showed non-significant results. When the ANOVA showed a difference between the treatments, Tukey's post-hoc analysis was performed to identify significantly different treatments at a 5 % significance level.

3. Results

3.1. Study of the algal red rust pathogen

3.1.1. Field observations and disease symptoms

During the field visit in each tea garden, circular, brick red colour with velvet-like texture, was observed on the adaxial surface of the leaf in Teenali cultivars. The epiphytic stage of the ARR pathogen appeared from thin, dull white or red discs with lobed margins, rising from a central point with rows of filaments (Fig. 1a). The parasitic phase infection towards the abaxial surface of leaves was also examined (Fig. 1b and c). During maturation, the disc produced brick red filaments containing sporangia enclosing several spores (Fig. 1c). Following the stem colonization (Fig. 1d), the leaves become hardened and cracked longitudinally. In the advanced stage of infection, ARR damaged young tea plants by deteriorating patches of stem tissue, resulting in dieback and complete defoliation of the tea bush (Fig. 1S).

3.1.2. Host invasion type study of Cephaleuros species

Microscopic examination of the infected plant parts revealed the presence of subcuticular algal filaments confined between the cuticle layer and the epidermal cells (Fig. 2a and b). Intercellular algal filaments were present beneath the epidermal cells and, sometimes, under the cuticle (Fig. 2 c and d).

3.1.3. Cultural and morphological characteristics of Cephaleuros species

The cultural and morphological features of both epiphytic and parasitic phases of the isolates were similar. No isolate of the pathogen grew on potato dextrose and Czapek's broth media; however, slow growth was observed on Trebouxia liquid medium. The colony colour of each isolate on Trebouxia agar medium was initially colourless and later changed to light green or dull white (Fig. 1 e and f). The radial growth of each isolate on Trebouxia agar medium was very slow, reaching in the range between 11.8 mm and 14.8 mm (Table 1).

The microscopic observations of the pathogens revealed the presence of sporangia and sporangiophores (Table 1). The sporangia length and width of subcuticular and intercellular isolates ranged from 22.4 to 37.7 × 17.4 to 19.6 μm (Fig. 3 a and b) and ranged from 27.8 to 39.4 × 19.8 to 24.6 μm (Table 1; Fig. 3 c and d), respectively. The sporangiophores, brick red to dark orange, were branched, septate, and arose either singly or in clusters (3–5) from the terminal cells of the ramuli, measured 337 to 548 × 12.6 to 20.1 μm for sub-cuticular isolates and 488.6 to 764.2 × 21.8 to 30.8 μm for intercellular isolates. The terminal head cells of the sporangiophores of subcuticular and intercellular isolates bore 4–9 (Fig. 3a and b) and 3–7 (Fig. 3c and d) sporangia, respectively. The sporangia were connected with sterigmata to the sporangiophores (Fig. 3 **a–d**). The host invasion and micro-morphological analysis revealed that subcuticular isolates NKR01, JITR01, SNYR01, SSR01, HTKR01, and intercellular isolates NKR02, JITR02, SNYR02, SSR02, HTKR02 were similar to each other. Therefore, only three isolates of each host invasion type were processed for molecular characterisation.

3.1.4. DNA sequencing and phylogenetic analysis of algal red rust pathogen

The sequencing analysis resulted in 1010 bp, 1012 bp, and 918 bp genes of SSU rRNA of isolates NKR01, JITR01, and SNYR01, and 1232 bp, 1155 bp, and 1185 bp of isolates NKR02, JITR02, and SNYR02, respectively. The BLAST analysis revealed that the SSU sequences of isolates NKR01, JITR01, and SNYR01 investigated in the present study resembled the highest similarity (>99.0) with *C. virescens* (MT705986.1), whereas isolates NKR02, JITR02, and SNYR02 shared the highest similarity (>99.0) with *C. parasiticus* (KM020146.1)

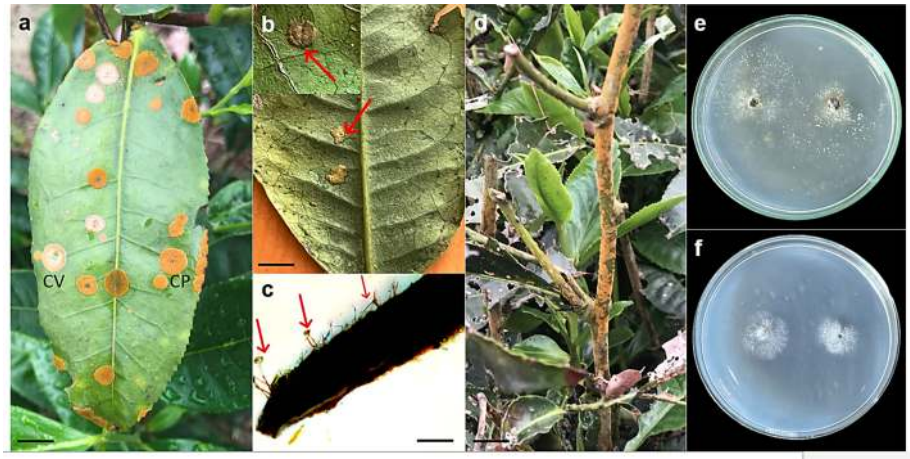


Fig. 1. Characteristic symptoms of algal red rust on tea (a. symptoms produced by *C. virescens* (CV, epiphytic phase) and *C. parasiticus* (CP, parasitic phase), b. lower leaf surface showing algal red rust spots (indicated with red arrow), c. sporangia on lower leaf surface (indicated with red arrow), d. colonization of *C. parasiticus* on the stem) and growth of *C. virescens* (e) and *C. parasiticus* (f) on Trebouxia agar medium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

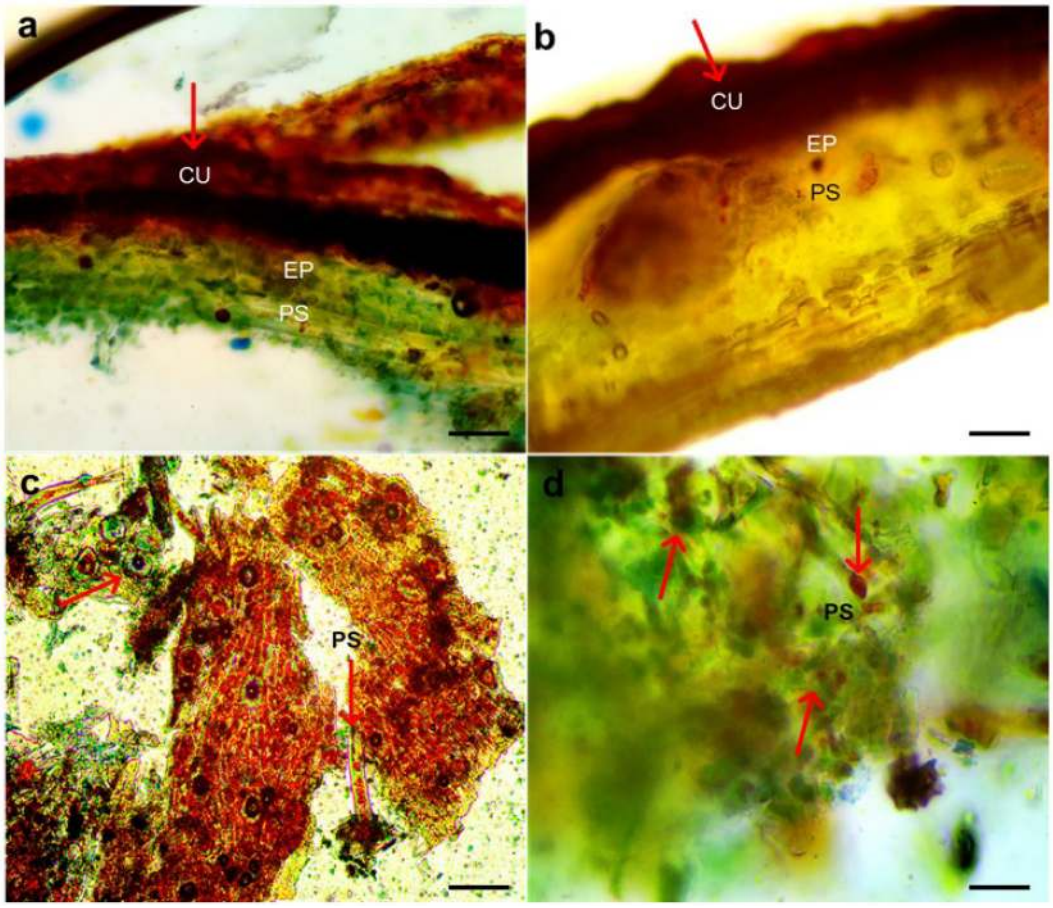


Fig. 2. Microscopical host invasion study showing two host invasion types of *Cephaleuros* sp. on tea plant, a and b represent subcuticular host invasion type where algae filaments are confined between the cuticle layer and the epidermal cells of isolates NKR01 and SNYR01, c and d indicate intercellular host invasion type isolates where algae filaments (shown with red arrow) are present beneath the epidermal cells/under the cuticle of isolates NKR02 and SNYR02; CU: cuticle layer; EP: epidermis and PS: Palisade cells.

Table 1
Morphological characteristics of *Cephaleuros* spp. isolated from the tea plant.

Isolates	Head cell*	Size (length × width) μm*		Number of septa in sporangiophores	Number of sporangia produced by each sporangiophore	Radial growth on Trebouxia agar medium (mm)
		Sporangia	Sporangiophores			
NKR0R01	26.5–54.6 × 20.2–53.5	22.4–35.5 × 17.4–19.6	348.2–540.3 × 13.6–19.3	4–9	2–7	13.5
NKR0R02	31.2–58.4 × 28.6–56.5	28.5–38.4 × 21.8–24.6	488.6–764.2 × 24.8–30.2	3–7	3–6	12.3
JIT0R01	24.3–52.8 × 18.5–48.6	27.8–37.4 × 16.8–19.3	337.8–498.9 × 12.6–19.1	5–8	2–6	14.8
JIT0R02	29.3–54.6 × 22.4–58.5	29.5–39.4 × 19.8–21.4	492.5–758.1 × 24.9–30.8	4–8	2–5	12.8
SNY0R01	26.8–53.9 × 23.2–51.4	24.5–36.4 × 17.8–19.5	349.4–548.3 × 14.7–19.8	4–6	3–6	14.2
SNY0R02	27.6–57.5 × 24.4–54.3	27.8–37.9 × 21.5–23.1	502.4–742.8 × 24.1–28.6	3–8	2–6	13.3
SSR01	24.8–53.1 × 20.3–51.9	26.8–36.9 × 18.8–19.3	340.4–541.9 × 13.9–19.5	4–6	2–6	13.3
SSR02	30.2–57.7 × 27.3–58.6	29.8–37.5 × 20.3–22.1	495.5–738.7 × 21.8–24.9	4–8	2–5	12.5
HTKR01	25.1–55.2 × 21.5–52.6	23.4–37.7 × 17.3–18.9	344.4–523.3 × 14.6–20.10	4–6	2–6	12.0
HTKR02	28.8–58.9 × 27.7–55.1	28.1–35.9 × 20.9–23.6	482.6–698.8 × 22.9–27.8	4–8	2–5	11.8

*Average of 20.

available in NCBI GenBank. The obtained sequences of all six isolates of ARR pathogens were submitted to GenBank under accession numbers provided in Table 2S. Based on the morphological and molecular analysis, isolates NKR01, JITR01, and SNYR01 were identified as *C. virescens,* and isolates NKR02, JITR02, and SNYR02 as *C. parasiticus*.

To check the overall tree topology of individual data, the obtained

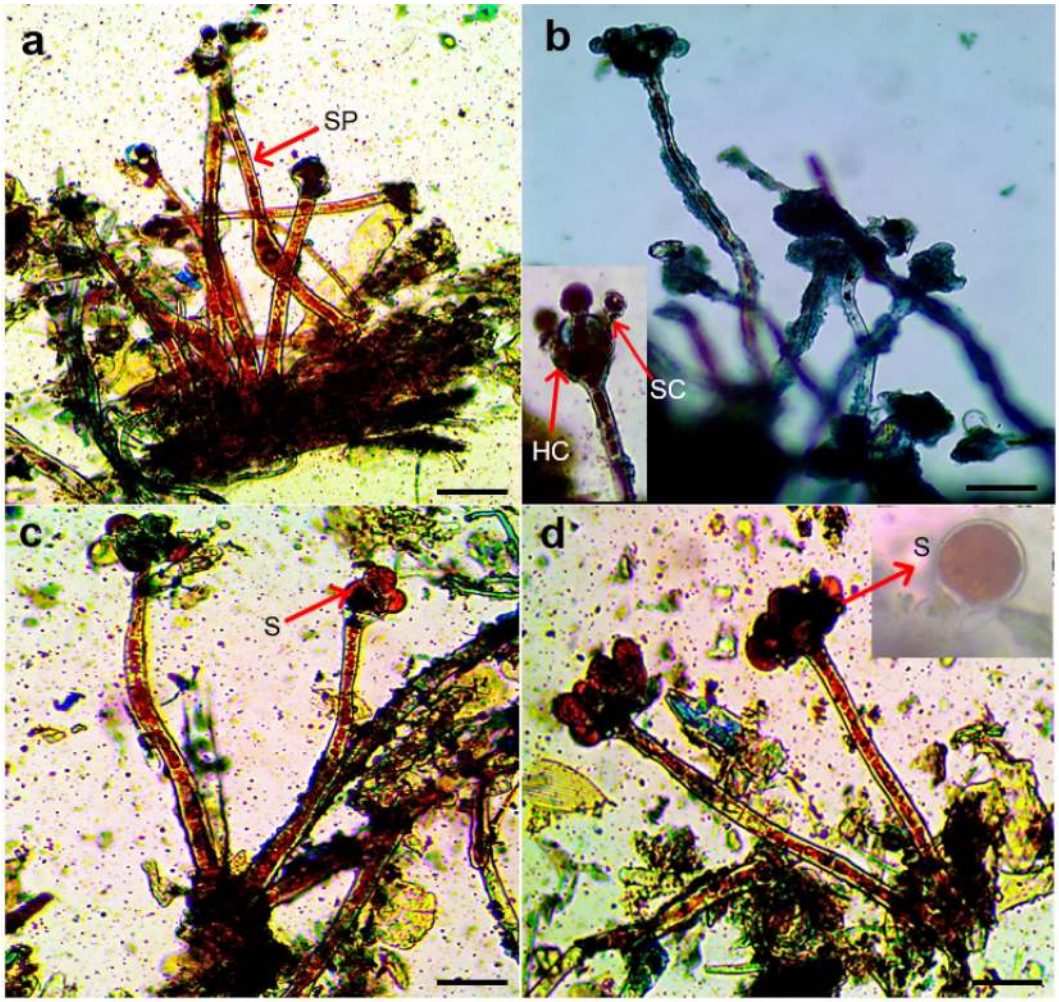


Fig. 3. Micro-morphological analysis of algal red rust pathogens *C. virescens* (a-NKR01 and b-SNYR01) and *C. parasiticus* (c-NKR02 and d-SNYR02) in tea; SP: Sporangiophores, HC: Head Cell, SC: Suspensor Cell, S: Sporangia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trees through Maximum Likelihood and Bayesian Inference were compared on a manual basis. The phylogenetic tree generated through the Maximum Likelihood method was consistent with the tree constructed through the Bayesian Inference method (Fig. 4**).** The Bayesian analysis provided 1000 trees after one million generations, following the topological convergence. In total, 250 trees showing the burn-in phase were removed from the analysis, and the remaining trees were used to compute posterior probabilities. Bayesian posterior probabilities showed higher phylogenetic support values than those of the Maximum Likelihood bootstrap (Fig. 4). In total, 8 strains, 4 each of *C. parasiticus* and *C. virescens,* were aligned on the tree, representing two clades of *Cephaleuros* species. The phylogenetic analyses led to two clades of the pathogen, with isolates NKR01, JITR01, and SNYR01 in one clade clustered with *C. virescens,* and NKR02, JITR02, and SNYR02 in another clade clustered with *C. parasiticus,* with a high bootstrap support of >99 %. The sequences of *Trentepohlia aurea*, used as an out-group, formed a single clade separating from all the isolates (Fig. 4). The phylogenetic trees also separated two clades of isolates based on their host invasion type: one clade of isolates NKR01, JITR01, and SNYR01 with subcuticular type host invasion, and another clade of isolates NKR02, JITR02, and SNYR02 with intercellular type host invasion (Fig. 4).

3.2. Rhizobacterial antagonists

3.2.1. Morpho-cultural characteristics

The colonies of isolate BOK01 were dull white with wrinkly to smooth surfaces and irregular shape (Fig. 5 a**),** measuring 2–4 mm. In contrast, isolate BPATH02 (Fig. 5 b) was slightly yellowish or grey-yellowish, measured 4–6 mm. Both isolates showed Gram-positive reaction, produced submerged spores in broth culture, and were catalase positive (Pandey et al., 2023b). Light and scanning electron microscopy observations revealed that both isolates were rod-shaped. However, isolate BOK01 had a bigger rod with cell size 2.19–3.19 × 0.78–1.29 μm (Fig. 5 c) than isolate BPATH02, which had a short rod with sizes in the range of 0.67–1.43 × 0.69–1.23 μm with an irregular arrangement (Fig. 5 d). Isolate BOK01 was spore-forming and motile, while isolate BPATH02 was non-spore-forming and non-motile.

3.2.2. Gene sequencing and phylogenetic analysis

Sequencing of the 16S rRNA gene of isolates BOK01 and BPATH02 produced 1475 bp and 1483 bp, respectively. The BLASTn analysis showed 99.7 % and 100 % similarity of isolate BOK01 with *Bacillus paramycoides* MT373523 and of isolate BPATH02 with *Microbacterium barkeri* NR026164, respectively. The phylogenetic analysis of the 16S rRNA gene of both isolates showed that isolate BOK01 was affiliated with *B. paramycoides* and isolate BPATH02 with *M. barkeri*, an actinobacterium (Fig. 2S). The outgroup sequence was completely separated

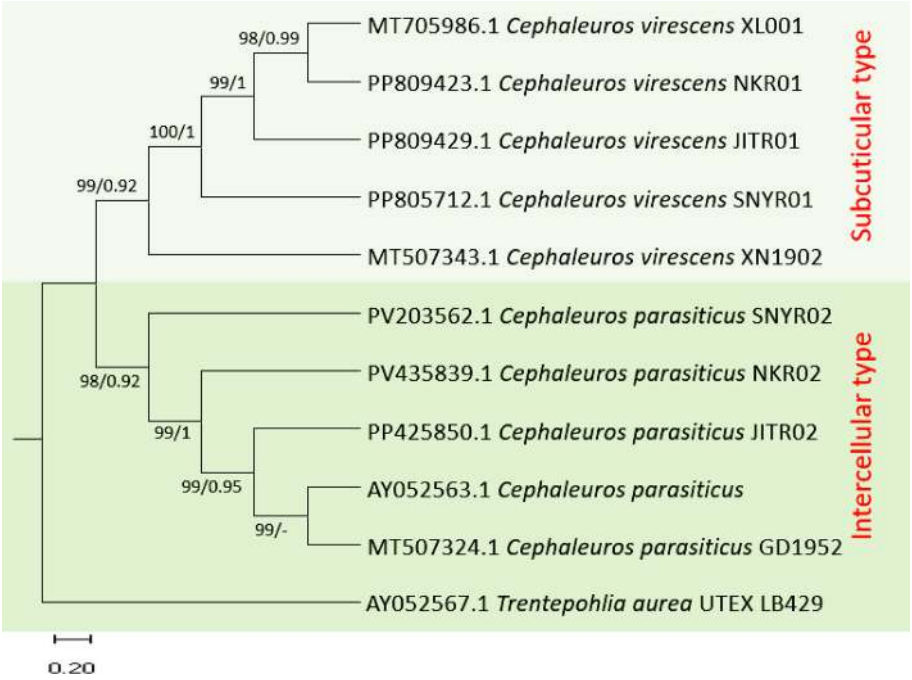


Fig. 4. Phylogenetic tree obtained using the ML method based on 18S small subunit gene sequences of 10 strains, showing the relationship of 5 each with *C. virescens* (Subcuticular type) and *C. parasiticus* (Intercellular type), and with the *Trentepohlia aurea* as the outgroup taxon. The numbers in the bootstrap test (500 replicates) are shown above and below the branches. Bootstrap support values for ML greater than 90 % are shown left at the nodes, and Bayesian posterior probabilities greater than or equal to 0.92 are indicated right at the nodes. The scale bar indicates the expected number of changes per site.

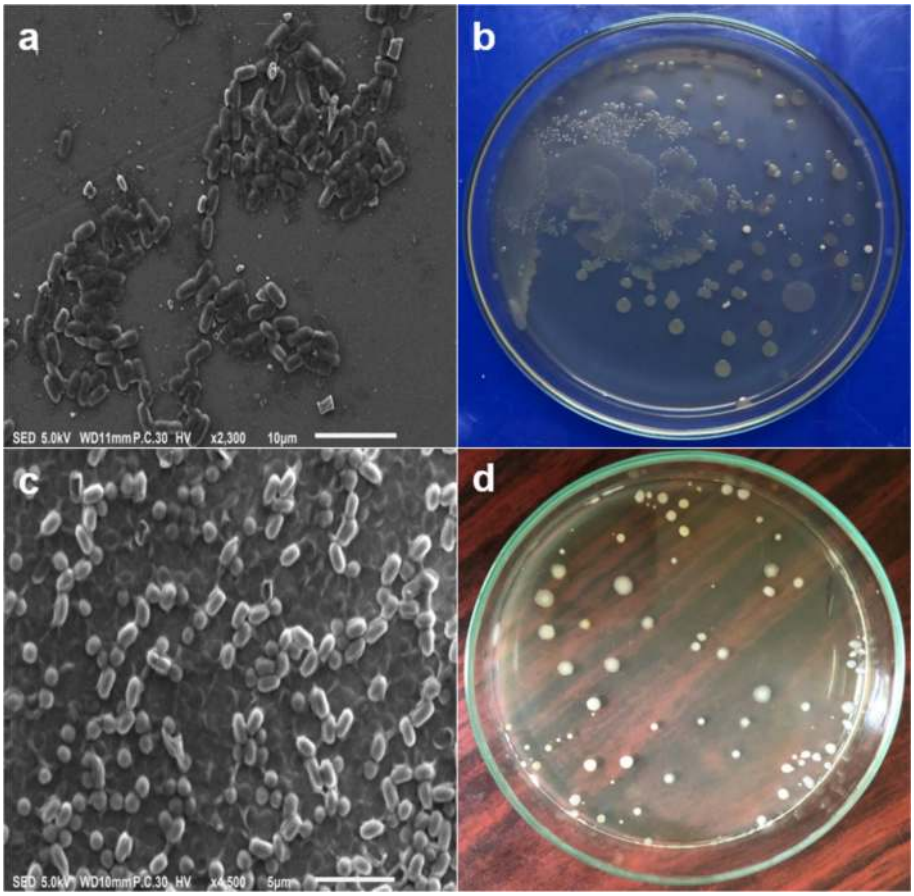


Fig. 5. Cell morphology under scanning electron microscopy (a and c) and cultural characters on nutrient agar plates (b and d) of *Bacillus paramycoides* BOK01 and *Microbacterium barkeri* BPATH02, respectively.

from both sequences.

3.3. Evaluation of bacterial antagonists in laboratory conditions

3.3.1. Efficacy of bacterial antagonists against Cephaleuros spp.

Both bacterial antagonists significantly reduced the spore viability of the *C. virescens* (CV 7.2, p = 0.0002) and *C. parasiticus* (CV 8.2, p = 0.0002) at 5 % concentrations. The isolate *B. paramycoides* BOK01 reduced 83.9 % of *C. virescens* and 84.6 % of *C. parasiticus* spore viability. The isolate *M. barkeri* BPATH02 reduced 79.8 % of *C. virescens* and 76.2 % of *C. parasiticus* spore viability at a 5 % concentration. For both bacterial antagonists, a significantly lower efficacy occurred with the reduction of concentrations **(Fig. 3S).** We found no statistically significant differences in the reduction of spore viability of *C. virescens* due to *B. paramycoides* BOK01 (p = 0.96), and *M. barkeri* BPATH02 (p = 0.81), and *C. parasiticus* due to *B. paramycoides* BOK01 (p = 0.38) at 2 % and 5 % concentrations, except the efficacy of *M. barkeri* BPATH02 at 2 % and 5 % concentrations against *C. parasiticus*. However, the spore viability reduction of the pathogens significantly differed at 1 % concentration between the bacterial antagonists and copper oxychloride (p = 0.0006). Copper oxychloride reduced the spore viability of *C. parasiticus* and *C. virescens* by 93.4 % and 87.03 %, respectively, with a significantly different effect from other treatments (p = 0.0002). Natural reduction in spore viability was also observed in control sets, with 10.2 % and 11.2 % reduction for *C. virescens* and *C. parasiticus*, respectively (Figs. 3S and 4Sa).

3.3.2. Evaluation of bacterial antagonists for their physiological properties

The melanin production intensity was significantly higher for isolate BOK01 on Peptone Iron and ISP7 agar Media compared to that of BPATH02. Both BOK01 and BPATH02 hydrolyzed starch, with a 26 mm and 24 mm halo zone, respectively. Both antagonists showed moderate to good growth at the temperature range of 15–35 °C, 48 h of post-incubation. However, a lower colony count was observed at 40 °C. Neither bacterial isolate grew at the lowest (5 °C) and highest (45 °C) temperatures tested during the study (Fig. 6 a), and their growth was statistically different (p = 0.0002) from other treatments (Table 2). A pH range 4-to-7 was found optimal for both bacterial growths, while fewer colony counts were reported at pH 2 and 9 (Fig. 6 b). Both BOK01 and BPATH02 grew on peptone broth supplemented with 8 % NaCl (Fig. 6 c).

3.4. Viability and stability of formulations

The viability and stability of each formulation, which were studied every month, began to reduce six months post-storage. For the *B. paramycoides* BOK01 formulation, the colony count significantly reduced (CV 7.67, p = 0.0002) from 131.7×10^8 CFU/ml to 0.7×10^8 CFU/ml after seven months post-storage. Likewise, the colony count in the *M. barkeri* BPATH02 formulation significantly reduced (CV 3.33, p = 0.0002) from 128.3×10^8 CFU/ml to 0.3×10^8 CFU/ml after seven months post-storage (Fig. 7a and b). The colony count reported seven months of post-storage of both bioformulations was below the CIBRC standard threshold of 1.0×10^8 determined for bacterial antagonists (CIBRC, 2025).

3.5. Evaluation of bacterial antagonists-based bioformulations under field conditions

3.5.1. Efficacy of bacterial antagonists-based formulations in the reduction of algal red rust disease

Both bacterial antagonist-based formulations were effective against ARR under field conditions. At each location and in each year, a 5 % concentration of both antagonist-based formulations significantly (p < 0.001) reduced disease compared to control. In 2021, higher disease reduction (bush recovery) was reported at NBRRDC, Nagrakata, than at Sannyasithan and Jiti tea gardens. At Nagrakata, 72.0 % and 69.4 % disease reductions **(Fig. 4Sb and c)** were achieved with 5 % concentration of *B. paramycoides* BOK01 and *M. barkeri* BPATH02, respectively, compared to the control. Disease reductions at 2 % concentration, i.e., 71.0 % with *B. paramycoides* BOK01 and 67.0 % (p = 1.1) with *M. barkeri* BPATH02 (p = 0.98), which were not significantly different from those achieved at 5 % concentration after the second spray. In contrast, disease reduction with both formulations significantly differed (p = 0.0002) at 1 % concentration compared to the control (Table 3; **Table 3S**). Similar results were reported at Jiti and Sannyasithan, with over 60.0 % disease reduction obtained with a 2 % concentration of each formulation after the second spray, compared to the control. This reduction in disease at Jiti (p = 0.94) and Sannyasithan (p = 0.14) did not significantly differ from that at 5 % concentration. Besides, 2 % and 5 % also differed from 1 % concentration for both antagonists (Table 3).

In 2022, the bacterial formulations showed higher disease reduction at Sannyasithan tea garden than at Jiti and Nagrakata. At Sannyasithan, 64.6 % and 63.4 % disease reductions compared to the control were achieved with 2 % concentrations of *B. paramycoides* BOK01 and *M. barkeri* BPATH02, respectively. These disease reductions did not statistically differ from those obtained with a 5 % concentration after the second spray, i.e., 68.0 % and 65.4 % for *B. paramycoides* BOK01 (p = 0.16) and *M. barkeri* BPATH02 (p = 0.42), respectively; Table 3). As for the other two gardens, higher disease reductions were reported at Nagrakata (i.e., 61.4 and 63.4 % with *B. paramycoides* BOK01, and 60.0 % and 61.4 % with *M. barkeri* BPATH02 at 2 % and 5 % concentrations, respectively). We found no significant difference in disease reductions at 2 % and 5 % concentrations of *B. paramycoides* BOK01 (p = 1.0) and *M. barkeri* BPATH02 (p = 0.98); disease reductions were significantly lower (p = 0.00) at 1 % concentration (Table 3). Similar results were reported at Jiti tea garden, where 2 % and 5 % concentrations of *B. paramycoides* BOK01 (p = 0.98) and *M. barkeri* BPATH02 (p = 0.99) formulations resulted in over 60.0 % disease reduction compared to the control after the second spray, with no significant differences between the two concentrations (Table 3).

In 2023, both bacterial antagonists-based formulations were further resulted in higher ARR control at Nagrakata, and were significantly (p = 0.0006) different from the disease reduction obtained at Jiti and Sannyasithan (Table 3). The 2 % and 5 % concentrations of *B. paramycoides* BOK01 provided 70.9 % and 72.6 % disease reductions, respectively, compared to the control, with no statistical difference (p = 0.95). However, disease reduction was significantly lower at 1 % concentration (i.e., 53.3 %) after the second spray (Table 3). The *M. barkeri* BPATH02-based formulation at 5 % and 2 % concentrations resulted in 70.2 % and 68.5 % disease reduction, respectively, compared to the control. Disease reductions at 5 % and 2 % did not significantly differ (p = 0.99) from each other, whereas they significantly differed from 1 % concentration (p = 0.0002; Table 3). At Sannyasithan, the *B. paramycoides* BOK01 and *M. barkeri* BPATH02-based formulations at 2 % & 5 % concentrations provided 68.3 % & 65.8 % (p = 0.94) and 69.3 % and 67.5 % (p = 0.99) disease reductions, respectively, compared to the control without a significant difference between them (Table 3). At Jiti, *B. paramycoides* BOK01 at 5 % and 2 % concentrations reduced the disease by 67.5 % and 66.8 %, respectively, compared to the control. The *M. barkeri* BPATH02-based formulation at 5 % and 2 % concentrations reduced the disease by 65.0 % and 63.3 %, respectively, compared to the control. No significant difference in disease reduction was found between the 2 % and 5 % concentrations of *B. paramycoides* BOK01 (p = 0.92) and *M. barkeri* BPATH02 (p = 0.35), whereas a significant (p = 0.00) difference in disease reduction was found at 1 % concentration (Table 3).

At each location in each year, copper oxychloride showed higher ARR reduction (from 68 % to 81 %, depending on the year and the locations) than the bacterial-antagonist-based formulations. The effectiveness of these two treatments was significantly different (p = 0.0002) from that of other treatments (Table 3). However, the *B. subtilis* commercial product showed lower disease reduction than *B. paramycoides* BOK01 and *M. barkeri* BPATH02-based formulations, and were

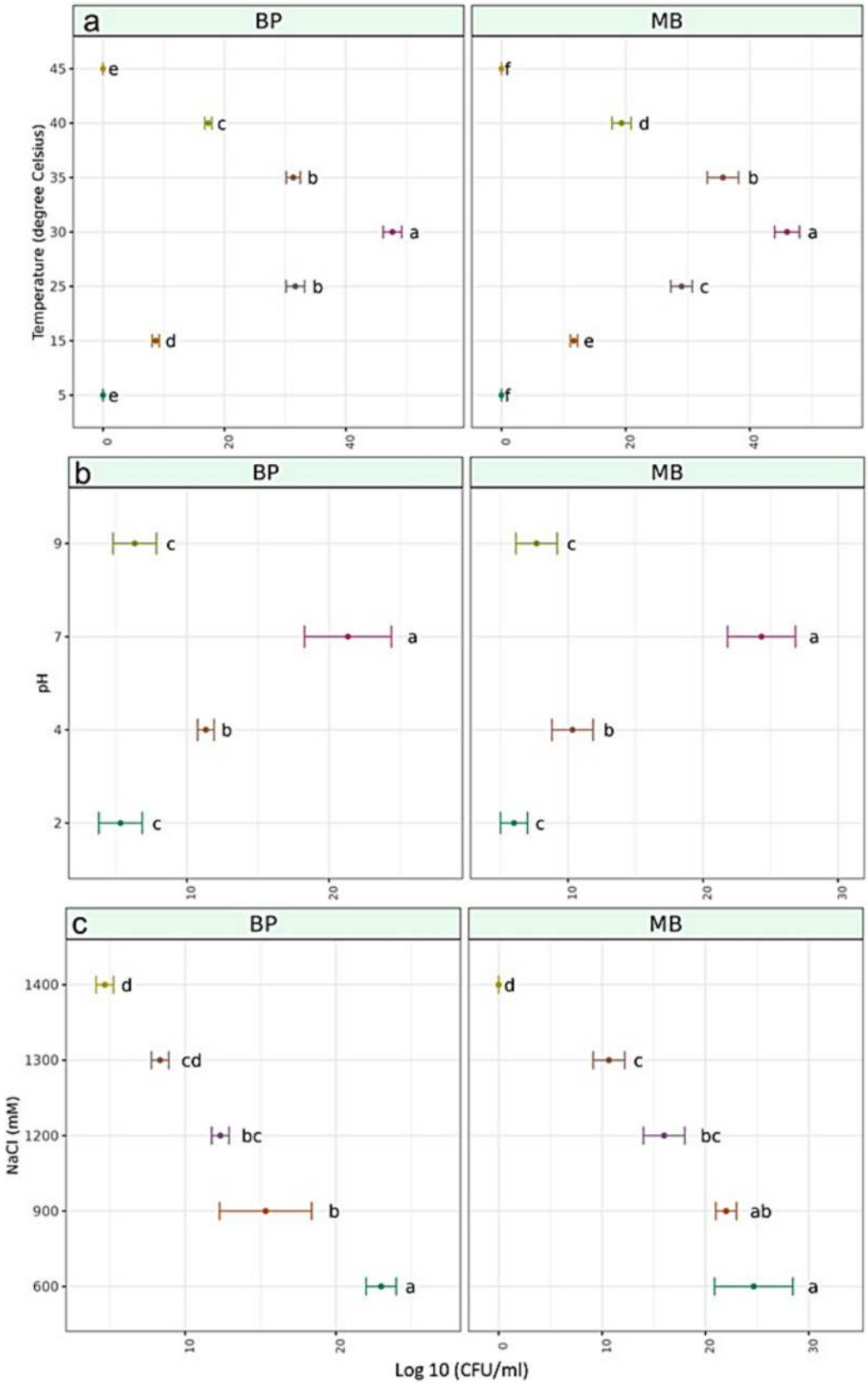


Fig. 6. Bar charts showing the effect of temperature (a), pH (b), and salinity (c) on the growth of *Bacillus paramycoides* BOK01 (BP) and *Microbacterium barkeri* BPATH02 (MB) in nutrient broths. Values with the same letters are not significantly different according to Tukey's least significant difference ($p < 0.05$).

Table 2
Analysis of variance on the effect of physical factors on the sporulation of bacterial antagonists.

ANOVA	*Bacillus paramycoides* BOK01			*Microbacterium barkeri* BPATH02		
	Temperature	pH	NaCl	Temperature	pH	NaCl
F stat	1359.67**	77.16**	63.70**	481.36**	235.84**	60.42**
HSD	2.43	4.08	4.29	3.98	2.66	6.18
MSE	0.72	2.08	2.32	1.94	0.89	4.80
SE(m)	0.49	0.83	0.88	0.81	0.54	1.26
CV (%)	4.35	13.02	11.95	6.89	7.80	14.94
Cohen's F	26.07	6.21	5.64	15.51	10.86	5.50

** indicates significance at $p < 0.01$, *indicated significance at $p < 0.05$.

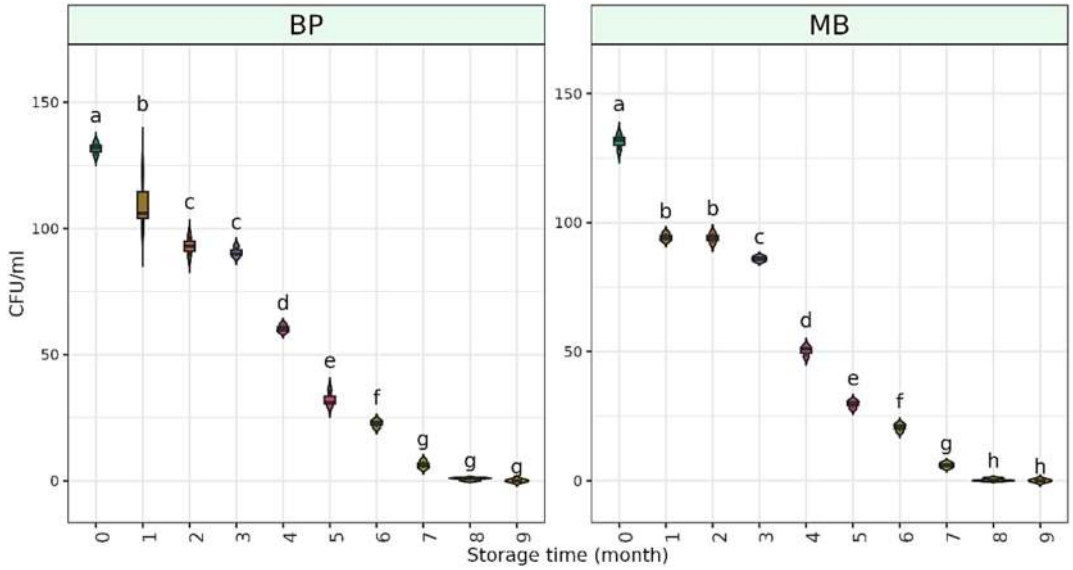


Fig. 7. Viability of formulations based on *B. paramycoides* BOK01 (BP) and *Microbacterium barkeri* BPATH02 (MB) after seven months of storage. Values with the same letters are not significantly different according to Tukey's least significant difference ($p < 0.05$).

significantly different ($p < 0.001$) from 2 % and 5 % treatments (Table 3) at each year and across locations.

3.5.2. Phytotoxic effect of bacterial antagonists-based formulations

None of the concentrations of the *B. paramycoides* BOK01 and *M. barkeri* BPATH02 formulations caused any phytotoxic effect on tea leaves and harvestable shoots. Both bacterial antagonists survived for over 4 months, although a significant reduction in bacterial population density occurred over time. The population density of *Bacillus* and *Microbacterium* ranged from 238.0 × 10^6 CFU/g to 7.7 CFU/g × 10^6 and from 196.3 × 10^6 CFU/g to 4.7 CFU/g 10^6 per leaf, respectively.

3.6. Crop yield

The average yields recorded from the first 6 pluckings at 7-day intervals across years and sites are presented in Table 4. The yield of control plots was significantly lower at each location in each year as compared to treatments with *B. paramycoides* BOK01 and *M. barkeri* BPATH02-based formulations and copper oxychloride. The green leaf yield was significantly ($p < 0.001$) higher in plots sprayed with 2 % and 5 % concentrations of *B. paramycoides* BOK01 and *M. barkeri* BPATH02-based formulations without a significant difference ($p = 0.88$) compared to copper oxychloride at each location and year (Tables 4 **and 4S**). The plots sprayed with a commercial product of *B. subtilis* showed lower green leaf yield compared to the studied bacterial formulations (Table 4).

4. Discussion

A correct identification of the causal agent causing ARR is a crucial step for sustainable disease management. Therefore, the present study was proposed to evaluate and identify potential bacterial antagonists for the sustainable management of tea ARR. *Cephaleuros virescens* has been reported as the causal agent of tea ARR in China (Fang et al., 2021), Ethiopia (Dechassa et al., 2020), and Queensland, Australia (Johnson and Hobman, 1982). In India, some authors associate only *C. parasiticus* (Ramya et al., 2013) with the disease, whereas others associate both *C. parasiticus* and *C. virescens* (Barthakur, 2011) as the causal agents of the disease. Based on the sampling of diseased tea plants across an important spatio-temporal gradient, followed by detailed morphological, physiological, pathogenicity, and molecular analyses, we revealed that both *C. parasiticus* and *C. virescens* are associated with tea ARR in India. Previous studies also used 18S Small Subunit markers to characterize *Cephaleuros* species from various hosts (Zhu et al., 2017), such as *C. virescens* from para rubber in Thailand (Pitaloka et al., 2015), and *C. parasiticus* from *Neoregelia* bromeliads in Florida, USA (Sanahuja et al., 2018). However, despite the absence of type strain sequences in GenBank, the 18S SSU rRNA gene alone does not provide enough resolution to confidently separate *Cephaleuros* species. Thus, the results of molecular analysis reported in this study may be treated as supplementary to morphological analysis. Besides, the shape and size of head cells, sporangia, and sporangiophores reported in this study were within the range reported by earlier investigators for *C. virescens* (Brooks et al., 2015; Muthukumar et al., 2015) and *C. parasiticus* (Suto et al., 2014).

The type of host invasion was the first distinctive feature used to differentiate *Cephaleuros* species into intercellular and subcuticular

Table 3
Algal red rust percent disease reduction under field conditions following application of the bacterial antagonist-based formulations.

Treatments	Nagrakata				Sannyasithan				Jiti			
	15 DAS		30 DAS		15 DAS		30 DAS		15 DAS		30 DAS	
	DSS*	PDR	DSS*	PDR	DSS*	PDR	DSS*	PDR	DSS*	PDR	DSS*	PDR
2021												
1 % BP	$2.87^{bc} \pm 0.19$	42.6	$2.70^{b} \pm 0.22$	46.0	$3.07^{bcd} \pm 0.09$	38.6	$2.80^{b} \pm 0.08$	44.0	$3.30^{cd} \pm 0.08$	34.0	$2.93^{b} \pm 0.19$	41.4
2 % BP	$2.43^{c} \pm 0.05$	51.4	$1.47^{c} \pm 0.19$	70.6	$2.57^{cd} \pm 0.21$	48.6	$1.63^{d} \pm 0.26$	67.4	$2.67^{g} \pm 0.21$	46.6	$1.87^{c} \pm 0.25$	62.6
5 % BP	$2.33^{c} \pm 0.19$	53.4	$1.40^{c} \pm 0.08$	72.0	$2.40^{d} \pm 0.16$	54.0	$1.57^{d} \pm 0.05$	68.6	$2.67^{g} \pm 0.09$	46.6	$1.73^{c} \pm 0.09$	65.4
1 % MB	$3.23^{b} \pm 0.21$	35.4	$2.50^{b} \pm 0.16$	50.0	$3.33^{bc} \pm 0.47$	33.4	$2.67^{bc} \pm 0.47$	46.6	$3.40^{c} \pm 0.16$	32.0	$2.97^{b} \pm 0.17$	40.6
2 % MB	$3.00^{bc} \pm 0.00$	40.0	$1.63^{c} \pm 0.21$	67.4	$2.87^{bcd} \pm 0.09$	22.6	$1.77^{cd} \pm 0.12$	64.6	$3.10^{de} \pm 0.08$	38.0	$1.97^{c} \pm 0.12$	60.4
5 % MB	$2.83^{bc} \pm 0.05$	43.4	$1.53^{c} \pm 0.12$	69.4	$2.73^{cd} \pm 0.09$	45.4	$1.70^{cd} \pm 0.22$	66.0	$3.00^{ef} \pm 0.00$	40.0	$1.93^{c} \pm 0.09$	61.4
2 % BSC	$3.33^{b} \pm 0.47$	33.4	$2.80^{b} \pm 0.16$	44.0	$3.67^{b} \pm 0.47$	26.6	$2.67^{bc} \pm 0.47$	46.6	$4.00^{b} \pm 0/00$	20.0	$3.00^{b} \pm 0.00$	40.0
2.5 g/L COC	$2.27^{bc} \pm 0.05$	54.6	$1.37^{c} \pm 0.12$	72.6	$2.37^{bcd} \pm 0.17$	52.6	$1.40^{d} \pm 0.08$	72.0	$2.47^{fg} \pm 0.12$	50.6	$1.03^{d} \pm 0.12$	79.4
Control	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.0$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0
2022												
1 % BP	$3.40^{bcd} \pm 0.43$	32.0	$2.87^{b} \pm 0.05$	43.0	$3.00^{c} \pm 0.16$	40.0	$2.73^{b} \pm 0.17$	45.4	$3.50^{c} \pm 0.08$	30.0	$2.90^{b} \pm 0.08$	42.0
2 % BP	$2.80^{cd} \pm 0.28$	44.0	$1.93^{c} \pm 0.09$	61.4	$2.63^{c} \pm 0.17$	47.4	$1.77^{c} \pm 0.12$	64.6	$3.07^{cde} \pm 0.09$	38.6	$2.00^{c} \pm 0.00$	60.0
5 % BP	$2.60^{d} \pm 0.33$	48.0	$1.83^{c} \pm 0.05$	63.4	$2.53^{c} \pm 0.25$	49.4	$1.60^{c} \pm 0.24$	68.0	$2.77^{de} \pm 0.12$	44.6	$1.87^{c} \pm 0.09$	62.6
1 % MB	$3.67^{bc} \pm 0.47$	26.6	$2.93^{b} \pm 0.09$	41.4	$2.97^{c} \pm 0.17$	40.6	$2.80^{b} \pm 0.16$	44.0	$3.13^{cd} \pm 0.19$	37.4	$3.00^{b} \pm 0.00$	40.0
2 % MB	$2.93^{cd} \pm 0.09$	41.4	$2.00^{c} \pm 0.08$	60.0	$2.73^{c} \pm 0.09$	45.4	$1.83^{c} \pm 0.17$	63.4	$3.20^{cd} \pm 0.16$	36.0	$2.00^{c} \pm 0.00$	60.0
5 % MB	$2.87^{cd} \pm 0.19$	42.6	$1.93^{c} \pm 0.09$	61.4	$2.80^{c} \pm 0.16$	44.0	$1.73^{c} \pm 0.09$	65.4	$3.07^{cde} \pm 0.09$	38.6	$1.97^{c} \pm 0.05$	60.6
2 % BSC	$4.00^{b} \pm 0.00$	20.0	$3.00^{b} \pm 0.00$	40.0	$4.00^{b} \pm 0.00$	20.0	$3.00^{b} \pm 0.00$	40.0	$4.00^{b} \pm 0.00$	20.0	$3.00^{b} \pm 0.00$	40.0
2.5 g/L COC	$2.83^{cd} \pm 0.05$	42.4	$1.60^{d} \pm 0.16$	68.0	$2.47^{c} \pm 0.12$	50.6	$1.43^{c} \pm 0.12$	71.4	$2.67^{e} \pm 0.19$	46.6	$1.50^{d} \pm 0.08$	70.0
Control	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0	$5.0^{a} \pm 0.00$	0.0	$5.00^{a} \pm 0.00$	0.0
2023												
1 % BP	$2.27^{cd} \pm 0.38$	42.2	$1.93^{c} \pm 0.09$	53.3	$2.77^{bcd} \pm 0.12$	30.75	$2.00^{bc} \pm 0.00$	50.0	$2.67^{abc} \pm 0.47$	33.3	$2.00^{bc} \pm 0.08$	50.0
2 % BP	$1.87^{d} \pm 0.09$	52.4	$1.20^{d} \pm 0.16$	70.9	$2.07^{cd} \pm 0.09$	48.25	$1.27^{d} \pm 0.05$	68.3	$2.33^{bc} \pm 0.34$	41.8	$1.33^{cd} \pm 0.09$	66.8
5 % BP	$1.83^{d} \pm 0.05$	53.4	$1.13^{d} \pm 0.09$	72.6	$1.97^{d} \pm 0.05$	52.50	$1.23^{d} \pm 0.12$	69.3	$2.00^{c} \pm 0.00$	50.0	$1.30^{cd} \pm 0.08$	67.5
1 % MB	$2.37^{cd} \pm 0.26$	39.7	$1.93^{c} \pm 0.09$	53.3	$2.87^{bcd} \pm 0.19$	28.25	$2.00^{bc} \pm 0.16$	50.0	$2.67^{abc} \pm 0.94$	33.3	$2.27^{b} \pm 0.38$	43.3
2 % MB	$2.20^{cd} \pm 0.43$	44.02	$1.30^{d} \pm 0.08$	68.5	$2.27^{cd} \pm 0.50$	43.25	$1.37^{c} \pm 0.17$	65.8	$2.47^{bc} \pm 0.21$	38.3	$1.47^{cd} \pm 0.09$	63.3
5 % MB	$2.10^{cd} \pm 0.36$	46.6	$1.23^{d} \pm 0.21$	70.2	$2.23^{cd} \pm 0.26$	44.25	$1.30^{c} \pm 0.08$	67.5	$2.30^{bc} \pm 0.14$	42.5	$1.40^{cd} \pm 0.16$	65.0
2 % BSC	$3.67^{ab} \pm 0.47$	6.6	$2.67^{b} \pm 0.47$	35.4	$3.67^{ab} \pm 0.47$	8.25	$2.67^{b} \pm 0.47$	33.3	$3.67^{ab} \pm 0.47$	8.3	$2.67^{b} \pm 0.47$	33.3
2.5 g/L COC	$1.77^{d} \pm 0.12$	54.9	$0.80^{cd} \pm 0.14$	80.6	$1.90^{bc} \pm 0.16$	52.50	$1.00^{cd} \pm 0.00$	75.0	$1.87^{c} \pm 0.25$	53.3	$0.77^{d} \pm 0.12$	80.8
Control	$3.93^{a} \pm 0.09$	0.0	$4.13^{a} \pm 0.17$	0.00	$4.00^{a} \pm 0.00$	0.00	$4.00^{a} \pm 0.00$	0.0	$4.00^{a} \pm 0.00$	0.00	$4.00^{a} \pm 0.00$	0.0

Disease severity scores (DSS) with the same letter grouping are not significantly different ($p < 0.05$); *Values are mean of three replicates ± Standard error, PDR: Percent disease reduction over control; BP: *Bacillus paramycoides*, MB: *Microbacterium barkeri*, BSC: *Bacillus subtilis* commercial; COC: Copper oxychloride; DAS: Day after spray.

types (Thompson and Wujek, 1997). A subcuticular *Cephaleuros* species invades under the surface of the host cuticle and grows between the cuticle and the epidermis, producing sporangiophores at the top of the cuticle. Whereas, an intercellular *Cephaleuros* species penetrates the epidermis and grows under the cuticle, epidermis, and inside the mesophyll and produces sporangiophores through the upper and lower leaf surfaces (Brooks et al., 2015). We showed that the type of host invasion was correlated with the phylogenetic analysis, where the isolates with the intercellular mode of invasion, namely NKR02, SNYR02, and JITR02 (*C. parasiticus*), were separated from subcuticular isolates, NKR01, SNYR01, and JITR01 (*C. virescens*). Our results corroborate those of Huang et al. (2023), who showed that the subcuticular host invasion type *C. virescens* and the intercellular *C. parasiticus* species complex are clustered in distinct clades (Huang et al., 2023). This suggests that the *Cephaleuros* host invasion type is a typical convergent with groupings through phylogenetic analysis, and may be an important criterion for their separation into different species.

Based on 16S rRNA gene sequencing, we identified the two bacterial isolates tested in our study as *B. paramycoides* (BOK01) and *M. barkeri* (BPATH02). The 16S rRNA genome has been previously used to identify *B. paramycoides* (Gu et al., 2023) and *M. barkeri* (Das and Pattnaik, 2024; Liu et al., 2012). The phenotypic features and measurements of shape and size through light and scanning electron microscopy reported in this study are in agreement with the previous findings on *B. paramycoides* (Qin et al., 2024) and *M. barkeri* (Mariola et al., 2024). Our results revealed that both bacterial antagonists can grow at high temperatures (40 °C), alkaline pH (9), and up to 1400 mM saline concentration. This means that *B. paramycoides* BOK01 and *M. barkeri* BPATH02 can be deployed in alkaline and saline soil, including in semi-arid tropics. *Bacillus* and *Microbacterium* species dwell in various ecological niches, explaining their ability to survive at higher temperatures, acidic soils, alkaline soils, and saline soils (Kaushik et al., 2012; Nair and Raja, 2018). The physiological attributes permit both bacterial antagonists to compete with the native pathogens when introduced into the soil in tea gardens (Habe and Uesughi, 2000). In addition, pH levels and salinity of the soil are decisive for the competition and survival of the bacterial agents. Thus, while selecting beneficial microbes against ARR pathogens, they must be evaluated for these traits, and this should be a basis for their selection criteria.

We showed that both bacterial antagonists used in this study have potential in reducing *Cephaleuros* spp. spore viability and their efficacy did not significantly differ from that of copper oxychloride. While ARR disease symptoms were frequently observed all year round on different tea cultivars in North Bengal tea gardens, the disease was particularly severe on cv. Teenali to both epiphytic and parasitic phases. The disease incidence was higher from June to September; hence, this period was selected for field evaluation trials. In the present study, the bushes recovered better in plots sprayed with formulations than in the control plots (Fig. 4Sb and c). The effectiveness of liquid formulations based on *B. paramycoides* BOK01 and *M. barkeri* BPATH02 in disease reductions was confirmed via field trials across a wide spatio-temporal gradient. We showed that 2 % and 5 % concentrations of formulations based on both bacterial antagonists significantly reduced ARR severity (recovery of tea bushes) compared to the control at each location across years, and with no significant difference with copper oxychloride. However, the higher green leaf yield was obtained with plots sprayed with bacterial antagonists-based formulations than with copper oxychloride. Similar results were found by Ramya et al. (2013), who reported that the efficacy of microbial antagonists (*Trichoderma* spp., *Pseudomonas fluorescens*, *Streptomyces* sp., and *Bacillus subtilis*) against tea ARR, although not significantly different from synthetic algicides, green leaf yield was higher with microbial agents (Ramya et al., 2013). This may be due to

Table 4
Effect of bacterial antagonist-based formulations on the tea yield across years and study locations.

Treatments	2021					2022					2023				
	Nagrakata		Sannyasithan		Jiti	Nagrakata		Sannyasithan		Jiti	Nagrakata		Sannyasithan		Jiti	
	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)	Yield* (kg/ plot)	Made tea yield (kg/ha)
1 % BP	$2.6^{c} \pm 0.20$	195.0	$2.6^{cd} \pm 0.12$	195.0	$2.4^{d} \pm 0.20$	180.0	$2.2^{c} \pm 0.06$	165.0	$2.4^{c} \pm 0.14$	180.0	$1.9^{b} \pm 0.06$	142.5	$2.7^{c} \pm 0.31$	202.5	$2.5^{c} \pm 0.31$	187.5
2 % BP	$4.07^{a} \pm 0.06$	307.5	$3.8^{a} \pm 0.16$	285.0	$3.5^{ab} \pm 0.12$	262.5	$3.5^{ab} \pm 0.31$	262.5	$3.7^{a} \pm 0.25$	277.5	$3.3^{a} \pm 0.31$	247.5	$4.2^{a} \pm 0.23$	315.0	$3.8^{a} \pm 0.23$	285.0
5 % BP	$4.03^{ab} \pm 0.06$	300.0	$3.9^{a} \pm 0.09$	292.5	$3.6^{a} \pm 0.15$	270.0	$3.6^{a} \pm 0.35$	270.0	$3.8^{a} \pm 0.05$	285.0	$3.3^{a} \pm 0.17$	247.5	$4.1^{ab} \pm 0.10$	307.5	$3.7^{a} \pm 0.10$	277.5
1 % MB	$2.7^{c} \pm 0.10$	202.5	$2.5^{de} \pm 0.08$	187.5	$2.2^{d} \pm 0.25$	165.0	$2.1^{c} \pm 0.23$	157.5	$2.3^{c} \pm 0.08$	172.5	$1.8^{bc} \pm 0.25$	135.0	$2.8^{c} \pm 0.06$	210.0	$2.4^{c} \pm 0.06$	180.0
2 % MB	$3.7^{ab} \pm 0.21$	277.5	$3.2^{b} \pm 0.21$	240.0	$2.9^{c} \pm 0.15$	217.5	$3.2^{ab} \pm 0.15$	240.0	$3.3^{ab} \pm 0.12$	247.5	$2.8^{a} \pm 0.20$	210.0	$3.8^{ab} \pm 0.26$	285.0	$3.7^{ab} \pm 0.15$	232.5
5 % MB	$3.6^{b} \pm 0.17$	270.0	$3.0^{bc} \pm 0.00$	217.5	$3.1^{bc} \pm 0.10$	232.5	$2.9^{b} \pm 0.12$	217.5	$3.0^{b} \pm 0.16$	225.0	$2.9^{a} \pm 0.10$	217.5	$3.9^{ab} \pm 0.12$	292.5	$3.3^{b} \pm 0.00$	247.5
2 % BSC	$1.7^{d} \pm 0.23$	127.5	$2.07^{ef} \pm 0.12$	157.5	$1.9^{de} \pm 0.12$	142.5	$1.9^{c} \pm 0.10$	142.5	$2.03^{cd} \pm 0.12$	150.0	$1.6^{bc} \pm 0.15$	120.0	$2.3^{cd} \pm 0.26$	172.5	$2.2^{de} \pm 0.12$	165.0
2.5 g/L COC	$3.9^{ab} \pm 0.10$	292.5	$3.3^{b} \pm 0.08$	247.5	$3.3^{abc} \pm 0.21$	247.5	$3.3^{ab} \pm 0.20$	247.5	$3.6^{a} \pm 0.16$	270.0	$3.1^{a} \pm 0.12$	232.5	$3.60^{b} \pm 0.17$	270.0	$3.7^{ab} \pm 0.15$	277.5
Control	$1.5^{d} \pm 0.20$	112.5	$1.8^{f} \pm 0.21$	135.0	$1.6^{e} \pm 0.15$	120.0	$1.7^{c} \pm 0.10$	127.5	$1.6^{d} \pm 0.14$	120.0	$1.3^{c} \pm 0.10$	97.5	$2.07^{d} \pm 0.12$	157.5	$2.0^{e} \pm 0.15$	150.0

Yield with the same letter grouping is not significantly different ($p < 0.05$); *Values are mean of three replicates ± Standard error, *Bacillus paramycoides*, MB: *Microbacterium barkeri*, BSC: *Bacillus subtilis* commercial; COC: Copper oxychloride.

the recovery of tea plants from ARR infection owing to increased metabolism by bacterial antagonists. The enhanced plant growth could also be due to plant growth-promoting attributes of both bacterial antagonists (Pandey et al., 2023a). A lower yield was recorded in the plots treated with copper oxychloride than with bacterial antagonists, which was not only due to the ARR reduction but also due to its phytotoxic effect (Sundaramoorthy et al., 2012).

We observed a higher disease severity (maximum score 5) in 2021 and 2022 than in 2023 at each location when treated with bacterial antagonists. ARR disease severity is governed by weather parameters such as rainfall, temperature, and RH, coupled with alkaline soil pH. The soil pH of all the test locations across our study sites ranged from 4.7 to 5.0, which is ideal for tea cultivation and less conducive for disease development (Barthakur, 2011). Temperature and relative humidity did not significantly differ across the years and locations, whereas rainfall patterns differed significantly. The differences in rainfall patterns during the study period could explain the higher disease pressure in 2021 and 2022, and less disease severity and better disease control in 2023 across all locations. Similar results were reported by Huq et al. (2022) in Bangladesh. Among the experimental sites, Sannyasithan was characterized by lower rainfall and relative humidity, but higher temperature than Nagrakata and lower than Jiti tea gardens during the investigation period. Microbial biopesticides work very effectively under low to moderate temperatures and high relative humidity, while high temperatures reduce the inoculum viability post-application (Wakefield, 2018). The higher disease pressure at Jiti than at Nagrakata and Sannyasithan across years may be due to differences in rainfall patterns, which help in the dispersal of zoospores.

Both bacterial-based formulations were found to be nonphytotoxic on tea even at higher concentrations. In contrast, phytotoxic effects of copper oxychloride have been reported in black tea (Karthika and Muraleedharan, 2009; Satheshkumar et al., 2011). Besides, residue levels of copper oxychloride above the European Union recommendation have also been reported in CTC black tea (Karthika and Muraleedharan, 2009; Satheshkumar et al., 2011). This implies that natural bio-algicides based on bacterial antagonists could be an alternative to reduce copper oxychloride use in tea gardens. This is possible via an integrated use of both bacterial antagonist-based formulations with synthetic chemicals at 15-day intervals that may help reduce the chemical residue level in the harvestable tea shoots (Liu et al., 2012). Besides, the enumeration results on the population density of bacterial antagonists revealed that both antagonists could consistently survive in sprayed leaf samples for more than six months. The post-application microbial survival in tea leaf samples has also been reported previously while studying other tea diseases (Gnanamangai and Ponmurugan, 2012; Sanjay et al., 2008). The competitive saprophytic ability of both bacterial antagonists, *B. paramycoides* (Yang et al., 2023) and *M. barkeri* (Liu et al., 2012), allows them to prolong survival in tea leaves and protect them from pathogen entry by serving as an effective barrier.

To date, few attempts have been made to deploy microbial biocontrol agents against the tea ARR disease. Here, we showed that liquid bacterial formulations could be an eco-friendly disease management strategy. The efficacy of *B. paramycoides* (Octarya et al., 2023) and *M. barkeri* (Liu et al., 2012) could be conferred by several volatile and non-volatile organic compounds. Further research should be conducted to identify the antialgal mode of action of these compounds against red rust pathogens, either in free form or as encapsulated compounds, followed by their commercialization. Before commercialization, both bacterial antagonist-based formulations must undergo regulatory risk assessment, including the potential effect on non-target organisms.

The shelf life is the main bottleneck of microbial biopesticides production and commercialization. During this study, sorghum extract was used as a carrier and source of nutrients for microbial growth, and yeast extract provided physical strength. In addition, carboxymethylcellulose was used as a thickener, stabilizer, and emulsifier to make the product viscous, stable, and textured and to prevent ingredients from separating.

The developed formulation supported the viability of bacterial antagonists for seven months of storage with $> 6.0 \times 10^8$ CFU/ml for both bacterial antagonists, which is above the CIBRC standard (CIBRC 2025). Klein et al. (2016) developed a talc-based formulation of *B. subtilis* added with 0.02 % urea and ammonium molybdate (1 nM), which enhanced shelf life and efficacy against *Colletotrichum acutatum,* causing post-bloom fruit drop of citrus. Muis and Quimio (2006) formulated a *B. subtilis*-based formulation added with 1 % brown sugar, 25 g inert carrier, 0.25 % yeast extract, and 1 % carboxymethylcellulose and reported higher CFU, i.e., 7.8×10^8 with six months of enhanced shelf life (Muis and Quimio, 2006). In the present study, the developed liquid formulations of both bacterial antagonists showed viability/shelf life up to seven months. The variations in viability may be due to different species of bacterial antagonists, their origin, and the formulation materials used during the study.

5. Conclusion

Based on the host-invasion and morpho-molecular analysis, we showed that *C. virescens* and *C. parasiticus* are the key pathogens causing ARR in tea in North Bengal. The liquid formulations based on bacterial antagonists, namely *B. paramycoides* BOK01 and *M. barkeri* BPATH02, isolated from the organic tea rhizosphere, possessed potential inhibitory activity against ARR pathogens both under laboratory and in field conditions, with more than six months of shelf life. Finally, through multilocation and multiyear field trials, we showed that the two bacterial-based liquid formulations provided the same yield performance as that of copper oxychloride, which is an encouraging result for farmers. However, a regulatory risk assessment must be conducted to ensure that these bacterial antagonist-based liquid formulations are safe for tea and non-target organisms, including humans, before their commercialization as bio-algicides.

CRediT authorship contribution statement

Abhay Kumar Pandey: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rajashri Saikia:** Methodology, Investigation, Formal analysis, Data curation. **Abhishek Kumar:** Methodology, Investigation, Formal analysis, Data curation. **Harshit K. Sharma:** Methodology, Investigation, Formal analysis, Data curation. **Somnath Roy:** Methodology, Investigation, Formal analysis, Data curation. **Jay Ram Lamichhane:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105918.

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