CELL BIOLOGY & DEVELOPMENT
Volume 4, Number 1, June 2020 E-ISSN: 2580-4499
Pages: 46-52 DOI: 10.13057/cellbioldev/v040105

Effect of antibiotics in eliminating bacterial wilt (*Ralstonia solanacearum*) from in vitro propagated ginger

TIGIST MARKOS, TILEYE FEYISSA♥
School of Graduate Studies, Institute of Biotechnology, Addis Ababa University. Addis Ababa, Ethiopia. Tel.: +251-11-1239706,
♥email: tileye.feyissa@aau.edu.et

Manuscript received: 22 March 2020. Revision accepted: 5 May 2020.

Abstract. *Markos T, Feyissa T. 2020. Effect of antibiotics in eliminating bacterial wilt (*Ralstonia solanacearum*) from in vitro propagated ginger. Cell Biol Dev 4: 46-52.* Ginger (*Zingiber officinale* Roscoe) is a herbaceous perennial. It is cultivated commercially in most tropical regions of the world and is a member of the Zingiberaceae family. In conventional ginger cultivation, underground rhizomes are used as planting material. However, it is afflicted by diseases that result in the senescence and degeneration of tissues. Due to *Ralstonia solanacearum* infection, substantial rhizome losses have been recorded. Therefore, disease-free planting material is required to cultivate ginger successfully. Plant tissue culture technology has been successfully used to commercialize pathogen-free plants and conserves the germplasm of rare and endangered species. In in-vitro, culture techniques offer an alternative method of plant multiplication and a method for crop enhancement. Frequently, ginger multiplication media are contaminated with *R. solanacearum*, which can survive endophytically in plantlets, rendering them useless for in vitro propagation. Therefore, an experiment was undertaken to determine the efficiency of antibiotics against the in vitro development of *R. solanacearum.* Four antibiotics, gentamicin, tetracycline, ampicillin, and streptomycin, at four concentrations, 130 mg/L, 160 mg/L, 200 mg/L, and 250 mg/L, were evaluated for their ability to eradicate bacteria from in-vitro propagated ginger. Gentamicin was proven superior to other therapies, with the maximum inhibition (22 mm) at 250 mg/L, followed by tetracycline at 200 mg/L (18 mm), streptomycin at 130 mg/L (13 mm), and ampicillin at 130 mg/L (12 mm). The *R. solanacearum* can be eradicated from in vitro propagated ginger by applying antibiotics in the micropropagation media.

Keywords: Identification, in vitro susceptibility test, inhibition zone, isolation

INTRODUCTION

Ginger is a perennial herbaceous, rhizomatous plant belonging to the Zingiberaceae family of the Scitaminae order. The genus Zingiber contains approximately 85 fragrant herb species. Ginger (*Zingiber officinale* Roscoe) is one of the most extensively produced fresh vegetables and dried spices from the Orient (George and Sivarana 2014). Ginger (Zingibil in Amharic) has been traded for longer than most other spices and may have been recognized in Ethiopia from ancient times; it is mostly farmed in the Southern Nations, Nationalities, and Peoples Regional State (SNNPRS). Some areas of Western Oromia and Northern Amhara have also begun producing ginger, albeit at a smaller level (Fikre and Kifle 2013).

Ginger thrives in warm, humid climates and is cultivated at altitudes between sea level and 1,500 meters above sea level. It can be grown in both rain-fed and irrigated environments. For the growth of ginger to be effective, there must be moderate precipitation from the time of seeding until the rhizomes sprout, fairly strong and evenly distributed rains during the growing period, and dry weather for about a month before harvesting. Ginger grows best in well-drained sandy loam, clay loam, red loam, or lateritic loam soils. A friable, humus-rich loam is suitable for growing ginger. However, because ginger is an exhaustible crop, it is not advisable to cultivate it annually on the same soil (Ravindran and Babu 2005). Conventionally propagated ginger rhizomes were contaminated with pathogens such as *Fusarium oxysporum*, *Pseudomonas solanacearum*, *Pythium* spp., and roundworms. As ginger is susceptible to soil-borne illnesses such as bacterial wilt, soft rot, and Fusarium wilt, the importation of fresh rhizomes for crop establishment, whether for study or commercial production, appears risky (Endrias and Kifle 2011).

In Ethiopia, pathogens are currently attacking ginger plants, and the disease's intensity is escalating (Hunduma et al. 2016). It is a result of the introduction of novel pathogen strains from different regions of the world via latently infected planting materials (George and Sivarana 2014). Because vegetative propagation is inefficient, several rhizomes are required for the typical vegetative propagation of ginger. In addition, rhizomes utilized for vegetative growth are sensitive to diseases that cause tissue senescence and degeneration during storage and cultivation. Heavy ginger losses have been seen due to *Ralstonia solanacearum*-caused bacterial wilt. The disease is transmitted primarily through rhizomes. Therefore, successful ginger gardening requires the production of disease-free clones. The most effective method for managing bacterial wilt is cultivating disease-free ginger from pathogen-free seed. In in-vitro, culture techniques offer an alternative method of plant multiplication and a method for crop enhancement. It has become an indispensable method for addressing the limitations of healthy plant material. Therefore, it is necessary to investigate ways to supply disease-free plant material. The

most effective methods for managing bacterial wilt involve the cultivation of disease-free ginger. Therefore, it must investigate the effect of drugs on eradicating *R. solanacearum* from ginger plantlets produced in vitro.

MATERIALS AND METHODS

Preparation of plant donors and stock solutions

Plant donor preparation: Ginger rhizomes were taken from the experimental site of the Areka Agricultural Research Center, located 300 kilometers southwest of Addis Ababa, Ethiopia, in the Wolayita Zone of the Southern Nations Nationalities and Peoples Regional state. Fresh rhizomes were properly cleansed with water and stored in sterile sand at 20 to 25 degrees Celsius in a greenhouse. The rhizomes were irrigated daily with distilled water for 15 to 20 days to induce sprouting. Explants derived from sprouted stem tips and auxiliary buds on these rhizomes were used in later tests.

MS stock preparation: As the basic components of the medium, Murashige and Skoog (MS) medium, with its complete macro and micronutrients, vitamins, sucrose, and agar, was utilized.

Preparation of plant growth regulators stock: The stock solutions of plant growth regulators (2.0 mg/L BAP and 1.0 mg/L kinetin) were made by weighing and dissolving the powder in double-distilled water at a concentration of 3 to 4 drops of 1 N HCl.

Culture medium preparation and culture condition

Proper amounts of MS stock solutions and 3% (w/w) sucrose were used to make the culture medium. The pH was adjusted to 5.8 with 1 N NaOH or 1N HCl, and a plant growth regulator was applied. Micro-oven was then utilized to dissolve 0.6% (w/v) agar. The medium was sterilized by autoclaving at 121°C for 15 minutes at 105 Kpa. Next, 40 ml of media was placed into a baby jar culture vessel for shoot initiation, multiplication, and rooting. Before closing, the culture vessel and its cap were flamed and sealed with a strip of Parafilm, and the vessels were properly identified. The maintaining cultures were 30 days, with a 12 h photoperiod and 3000 Lux of light intensity from cool white fluorescent lights at a temperature of 25+2°C.

Surface sterilization of explants and initiation of shoot

After storing new rhizomes in a greenhouse for 15 to 20 days at 20 to 25 degrees Celsius, the sprouting branch tips and auxiliary buds were employed as explants. First, the shoot tip and auxiliary bud explants were rinsed with sterile distilled water and then briefly submerged in 70% ethanol. Next, the explants were treated for 15 minutes under aseptic conditions with 5% active chlorine concentration local bleach (Clorox) containing two drops of Tween-20. The explants were then carefully cleaned (three to four times) with sterile distillate water. Around 0.7 to 1.5 cm long shoot tips were employed for shoot initiation. The explants were grown on MS medium supplemented with 2.0 mg/L BAP and 1.0 mg/L Kin.

Multiple shoot development, rooting, and acclimatization

Transferring the started shoots to MS media supplemented with 4.0 mg/L BAP for 30 days. Next, the multiplied shoots were cultured in MS Full strength medium for root formation with 1.0 mg/L NAA. The number of surviving plants was then recorded after the plantlets were planted in a sterilized standard potting mixture containing soil, sand, and coffee husk in a 3:1:2 ratio.

Isolation of bacterial contamination and characterization of the isolated bacterium

The lowest portions of well-regenerated plantlets were cut off, and the upper portions were suspended for ten minutes in five milliliters of sterile distilled water to extract detectable bacterial pollutants (*R. solanacearum*). The bacterial suspension was serially diluted with 9.0 mL of sterile water once the water in the test tube turned cloudy due to leaking bacterial cells from sick tissue. Bacterial ooze was obtained from pseudo-stems of infected plants by serially diluting the bacterial suspension in sterile distilled water and cultured on a TZC medium (Kelman 1954). The bacterial suspension was poured over the surface of Nutrient Agar (NA) using the serial dilution method and the streak plate method. The plates were then incubated for 24 hours at 28 degrees Celsius. Kelman (1954) described that *R. solanacearum* isolates were purified by streaking a single colony of each isolate on Triphenyl Tetrazolyl Chloride (TZC) medium and incubating the plates at 30°C for 36 hours.

Preparation of inoculums and Testing for Pathogenicity

For inoculation, a single colony of *R. solanacearum* exhibiting virulence, fluidity, irregularity, and creamy white with pink in the center was chosen and adjusted to 3.2 x 108 cfu mL^{-1}. Eight-leafed tissue-cultured Ginger plantlets were inoculated via stem piercing and leaf infection pinpricks. The bacterial suspension was sprayed on each test plant's two leaves (Stromberg et al. 2004). In a greenhouse, the virulence characteristics of *R. solanacearum* isolates were evaluated on healthy tissue culture plantlets. The seedlings were transferred into 9-centimeter-diameter plastic pots containing sterilized soil, sand, and coffee husk in a ratio of 1:1:2. They were then grown in the greenhouse. For inoculation, a single colony of *R. solanacearum* exhibiting virulence, fluidity, irregularity, and creamy white with pink in the center was chosen and adjusted to 3.2 x 108 cfu mL^{-1}. Ginger seedlings were inoculated with one milliliter of inoculums per plant via stem puncture and leaf infection pinpricks. The eighth-leaf stage plants were inoculated once by puncturing the stem's base with a needle soaked in inoculums. Each experiment was carried out thrice. The daytime and nighttime temperatures ranged from 25 to 35°C, with 12 hours of daylight and darkness. Symptoms of the disease were monitored for one week following vaccination. If the plant had symptoms of wilting, the Interaction was deemed pathogenic.

Isolates of bacteria in an antibiotic medium

Evaluation of antibacterial agents on the growth of R. solanacearum *in vitro*

Antibiotic sensitivity testing was conducted on bacterial isolates grown in Muller Hinton agar containing antibiotics (17.5 gm of Acid Hydrolysate of Casein, 2.0 gm of Beef Extract, 1.5 gm of Starch, 17.0 gm of agar). Four antibiotics, Gentamicin, Tetracycline, Ampicillin, and Streptomycin, were employed at various concentrations (130 mg/L, 160 mg/L, 200 mg/L, and 250 mg/L). Filter paper discs (Whatman no-44) measuring 6mm in diameter were soaked in the various chemical concentrations for 5 minutes before being transferred to the Muller Hinton medium in the Petri dishes. The inoculation plates were refrigerated for four hours at 4°C to permit the diffusion of chemicals into the medium. Then, the plates were incubated in the dark for 24 hours at 25°C and 25°C. The sizes of the inhibition zones surrounding the disks were measured and recorded.

Using antibiotics to treat plant material

The efficiency of Gentamicin, Tetracycline, Ampicillin, and Streptomycin in eradicating bacterial contamination was evaluated by this medicine. First, the 0.20-micrometer filter-sterilized membrane used was newly manufactured, and the antibiotics were added to the BAP-supplemented multiplication medium. Next, growing shoots were carefully excised along with the lower sheath base portion and transferred to a multiplication medium supplemented with 4.0 mg/L BAP and respective antibiotics. The medium was then incubated for 10 days under a 12 h photoperiod, 3000 Lux of light intensity from cool white fluorescent lamps, and a temperature of 25+0.5°C.

Data analysis

The experiment employs a CRD design in which one ginger variety (boziab) and four antibiotics with four concentration levels were utilized for four replicated treatments. The experimental data were analyzed using a one-way analysis of variance and a GLM comparison of means at the 0.05 significance level. Using Minitab 17.0, the mean, standard deviation, and standard error of the mean were evaluated. Compared to the average number of in-vitro-grown shoots, the growth components and elimination of pollutants status were studied.

RESULTS AND DISCUSSION

The initial surface sterilization experiment was effective when 0.7-1.5 cm shoot tips were treated with 70% ethanol for 5 minutes, followed by double sterilization with 5% active chlorine concentration of local bleach (Clorox) for 15 minutes. This procedure yielded 95% sterile explants and 2% dead explants 10 days after inoculation in shoot induction of MS media. When the shoots began to multiply, however, indicators of wilting emerged. Shoot tips were frequently contaminated after a few generations of culturing using plant material from the infected field.

MS medium contamination appeared as a hazy, yellowish-white color or was restricted to the soil surrounding the plant's root zone (Figure 1).

However, because the growth medium employed in tissue culture may not suit bacteria or inhibit their growth, pathogens may be undetected for lengthy periods (Isenegger et al. 2003).

On Tetrazolium Chloride (TZC) Agar selective media, isolates displayed a light to red color with a distinctive red center, similar to the pathogen *R. solanacearum.* All *R. solanacearum* isolates were shown to be virulent after 48 hours of incubation on TZC medium, yielding a pink or light red color or the unmistakable red core and white margin. On TZC media, virulent colonies appeared well-separated, irregular, fluid, and dull white with a small pink center (Figures 2.A and 2.B). In contrast, non-virulent colonies appeared dark red (Figure 2.C).

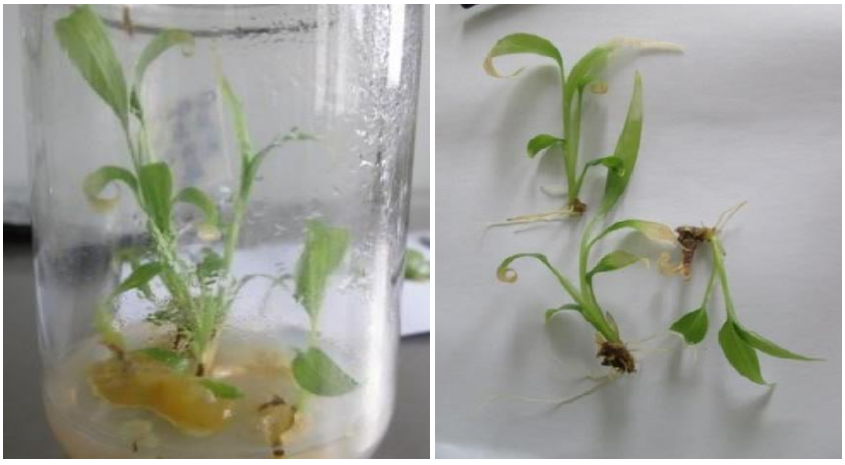

Figure 1. Diseased ginger plantlets in vitro

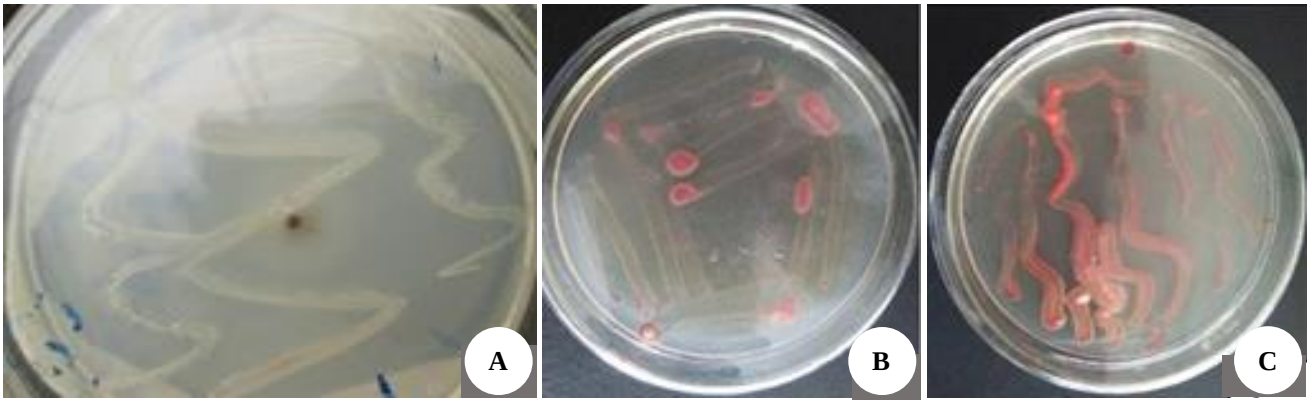

Figure 2. The *R. solanacearum* isolates on Tetrazolium Chloride (TZC) medium. Virulent colonies (A and B) and non-virulent colonies (C)

Pathogenicity for *Ralstonia solanacearum*

Pathogenicity test result for *R. solanacearum* within 5 to 15 days after inoculation, run isolation under artificial stem inoculation method caused ginger to wilt (Figure 3.A and 3B). The results of the pathogenicity study in India indicate that ginger wilt occurs between 5 and 7 days after inoculation (Kumar and Sarma 2004). Small, water-soaked lesions accompanied by leaf curling were observed as a symptom. The plants first exhibited daytime wilting and nighttime recovery, but after four to five days, they unexpectedly collapsed. The first wilt symptoms observed on the above-ground portion of the ginger plant were a mild yellowing and withering of the lower leaves. Then, the wilt spreads upward, affecting the younger leaves, followed by the yellowing and browning of the entire pseudo-steam. However, the plant dries out very quickly, and its leaves become yellow-brown within 5 to 10 days.

Young succulent shoots frequently become soft and rotting as pseudo-steam progresses, and diseased shoots easily separate from the underground rhizome at the soil line (Figures 3.A, 3.B, and 3.C). Kumar and Sarma (2004) described the identical ginger bacterial wilt symptom. The inoculated plant lost turgidity; leaves began falling, and the plant rapidly wilted (Figure 3.C). Initial developmental stage symptoms on leaves, pseudo-stems, and rhizomes of infected ginger plants were identical to those described in the literature (Trujillo 1964). Fine milky white filaments, composed of a mass of bacteria in extracellular slime, flow downward from the severed extremities of xylem arteries in vascular tissue. Bacterial exudates and leaf symptoms separate this wilt from fungus (Hayward 1964).

In vitro evaluation of antibacterial chemicals on the growth of *R. solanacearum*

This study was conducted to determine the efficacy of commercially available antibacterial agents against the growth of *R. solanacearum* under in vitro conditions. The findings of antibiotic susceptibility tests were derived from the data supplied by the disc diffusion method. In terms of zones of inhibition around the discs, the disc diffusion method for determining antibacterial activity revealed a considerable decrease in bacterial growth (Figure 4).

According to the activities of the four antibiotics on each bacteria, the inhibition zones grew as the antibiotic concentrations rose, indicating concentration-dependent activity. *R. solanacearum* isolates were highly susceptible to tetracycline, streptomycin, gentamycin, and ampicillin based on this study's antibiotic susceptibility testing of single antibiotic treatments. The *R. solanacearum*-inoculated Mueller-Hinton agar (MHA) medium is surrounded by discs carrying various antibiotics at varying doses.

Figure 3. After 6 to 8 days of inoculation with *R. solanacearum* (A and B), the inoculated plant exhibited wilting symptoms 15 days after inoculation (C)

Rooting and acclimatization

The experiment conducted in a greenhouse revealed that only 10% of plants survived when ginger plantlets were not treated with antibiotics. But ginger plantlets treated with antibiotics (antibiotics are added to the rooting medium before acclimation) decreased the incidence of bacterial wilt and enhanced its survival rate by 94% (Figure 5).

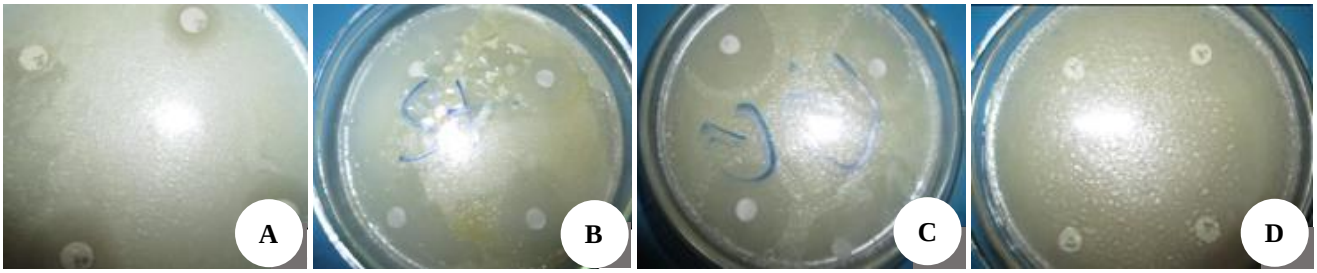

Figure 4. *Ralstonia solanacearum*-inoculated Mueller-HInternationalon agar (MHA) medium surrounded with discs containing various antibiotics at different doses. Streptomycin-containing discs (A and B), gentamicin-containing discs (C and D)

Figure 5. Acclimatized ginger plantlets treated with antibiotics (A), control (B)

Table 1. Inhibitory zones (mm) around discs containing different concentrations of various antibiotics placed on the surface of Mueller-Hinton agar (MHA) medium inoculated with the *Ralstonia solanacearum*

Anti-biotics	Concentration (mg/L)	Inhibitory zones (mm)
Ampicillin	130	10
	160	12
	200	5
	250	6
Tetracycline	130	12
	160	10
	200	10
	250	15
Gentamicin	130	13
	160	15
	200	18
	250	22
Streptomycin	130	6
	160	14
	200	12
	250	11
Antibiotics free	0	0

Table 2. Antibiotics treatment of plant material results in shoots free from *R. solanacearum*

Antibiotics	Antibiotics Concentration (mg/L)	Shoot number Mean±SD
Control	0	8.00±2.71abc
Ampicillin	130	11.51±1.29a
Ampicillin	160	8.75±1.70abc
Ampicillin	200	6.74±1.70bc
Ampicillin	250	7.00±1.40bc
Gentamaycine	130	6.00±1.40c
Gentamaycine	160	5.70±1.50c
Gentamaycine	200	5.75±1.70c
Gentamaycine	250	6.75±0.50bc
Streptomycin	130	6.75±1.25bc
Streptomycin	160	7.00±0.00bc
Streptomycin	200	6.60±0.57bc
Streptomycin	250	6.50±0.57bc
Tetracycline	130	8.00±1.5bc
Tetracycline	160	7.70±0.81bc
Tetracycline	200	7.20±0.50bc
Tetracycline	250	6.00±1.15c

Note: Numbers are mean and SD of Four replicates (four plants in each culture jar). Means followed by the same letter in a column are not significantly different by Tukey's test at $\alpha = 0.05\%$

Discussion

In the current investigation, diseased samples were gathered to identify the pathogens related to disease in in-vitro-produced ginger plantlets. The causative agent *R. solanacearum* was identified from the pseudo stem of ginger plantlets produced in vitro that exhibited the disease's distinctive symptoms. Following isolation, *R. solanacearum* isolates were purified by streaking a single colony of each isolate on a Tetrazolium chloride (TZC) agar medium. A single colony of *R. solanacearum* is virulent, fluid, uneven, and cream-colored with a pink center. In ginger plantlets produced in vitro, virulent isolates of *R. solanacearum* were found. On TZC medium, colonies of pink or light red color, or colonies with a red center and a whitish edge, were generated by *R. solanacearum* strains isolated from in vitro-cultivated ginger. After 24 hours of incubation, *R. solanacearum* formed colonies on TZC media that were fluid and pink or light red. These findings concur with those of Kelman (1954), Schaad et al. (1980), Suslow et al. (1982), French et al. (1995), and de Melo et al. (1999). The tentative identification of *R. solanacearum* was confirmed by the observation that none of the isolates preserved violet color, i.e., the isolates retained counter stain (pink color). These outcomes parallel those discovered by Suslow et al. (1982), who observed that the isolates of *R. solanacearum* did not preserve violet color, i.e., they retained counterstain (pink color).

The biochemical characterization demonstrated that the disease that threatens ginger under in vitro conditions is caused by *R. solanacearum* and that the disease was detected in the tissue of ginger plantlets. Furthermore, the etiology and pathogenicity tests confirmed that *R. solanacearum* causes bacterial wilt in ginger.

Pathogenicity tests conducted on an *R. solanacearum* isolate using artificial stem inoculation demonstrated that it caused wilt symptoms in ginger plants 7 to 15 days after inoculation. Several researchers have demonstrated the pathogenicity of *R. solanacearum*, which causes bacterial wilt (Winstead and Kelman 1952; Schell 2000; Williamson et al. 2002; Kumar and Sarma 2004; Umesha et al. 2005; Hikichi et al. 2007; Artal et al. 2012; Thomas and Upret 2014; Zulperi et al. 2014).

To recover healthy plants, knowledge of the effect of antibiotics on bacteria and plants is vital. By determining the minimal inhibitory concentration (MIC) and, subsequently, the minimal bactericidal concentration (MBC) of all stable antibiotics after 24 h of incubation following the antibiotic susceptibility test, the effect of the selected antibiotics on *R. solanacearum* isolates was determined. MBC reflects the cidal potential of the antibiotics on the isolates, whereas MIC reveals the inhibitory potential. According to Bonev et al. (2008), the efficiency of antibiotics can be measured by their ability to inhibit bacterial growth, as represented by the minimal inhibitory concentration (MIC), or by their ability to kill bacteria, as described by the minimal bactericidal concentration (MBC).

According to antibiotic sensitivity testing, gentamicin, tetracycline, streptomycin, and ampicillin were the most effective antibiotics against the growth of *R. solanacearum* in vitro. Bacterial growth was reduced when shoot tips were grown on an antibiotic-supplemented multiplication medium for three weeks. Intensive development of high-quality shoots also occurred. The shoot in antibiotic-grown cultures was truly devoid of bacterial contamination, and cultures have grown on MS medium without antibiotics. Thus, all of the tested antibiotics proved effective against *R. solanacearum*. These results are consistent with those previously reported by some researchers (Hidaka and Murano 1956; Dutta and Verma 1969; Indersenan et al. 1981; Khan et al. 1997; Singh et al. 2000; Devanath et al. 2002; Dubey 2005; Sunder et al. 2011; Gupta and Razdan 2013; Owoseni and Sangoyomi 2014).

Different quantities of streptocycline inhibited the growth of the pathogen, with a maximum inhibition zone (IZ) of greater than 25 mm at a concentration of 400 ppm. Paul (1998) observed that 250 and 500 ppm concentrations of ambistryn, oxytetracycline, and streptocycline inhibited *R. solanacearum* in vitro. Singh and Jagtap (2017) also documented the effectiveness of antibacterial chemicals and bioagents against the in vitro growth of *R. solanacearum.* The average inhibition ranged from 6.2 mm (copper hydroxide) to 20.05 mm (Streptocycline). However, the average inhibition was much greatest for streptocycline (20.05 mm). Then, the medicines gentamycin (17.5 mm), tetracycline (16.5 mm), and Streptocycline (11.95 mm) were administered.

In conclusion, according to the study's results, the R. solanacearum survived surface sterilization of ginger explants before their use in tissue culture laboratories. Antibiotics will lower the pollutants, increasing plantlets' survival rate as the prevalence of *R. solanacearum* in plant tissue culture continues to rise. The *R. solanacearum* continues to pose a concern to plant tissue culture. However, strategies for minimizing pollutants with antibiotics have shown that gentamicin is substantially more effective than other treatments, with the greatest inhibition, followed by tetracycline and streptomycin. Antibiotic therapy of plants could eradicate bacterial contamination from in vitro plantlets that have been infected. Therefore, the information here suggests that chemical control of the bacterial wilt of ginger may be possible. Under laboratory circumstances, the antibiotics could eradicate the bacterium in this experiment.

REFERENCES

Artal RB, Gopalakrishnan C, Thippeswamy B. 2012. An efficient inoculation method to screen tomato, brinjal and chilli entries for bacterial wilt resistance. J Hortic Crops 18 (1): 70-73.

Bonev B. Hooper J, Parisot J. 2008. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Antimicrob Chemother 61 (6): 295-301. DOI: 10.1093/jac/dkn090.

de Melo MS, Furuya N, Iiyama K, Khan AA, Matsuyama N. 1999. Geographical distribution of biovars of *Ralstonia solanacearum*. J Agric 44: 9-15. DOI: 10.5109/24300.

Devanath HK, Pathank JJ, Bora LC. 2002. In vitro sensitivity of *Ralstonia solanacearum* causing bacterial wilt of ginger towards antagonists, plant extracts and chemicals. J Intl Acad 6 (2): 250-253.

Dubey SC. 2005. Intergrated management of bacterial wilt of tomato. Plant Dis Res 20 (1): 52-54.

Dutta AK, Verma SSP. 1969. Control of bacterial wilt of egg plant with streptocycline. Antibiotics Bull 11: 260-261.

Fikre T, Kifle A. 2013. Production, Post harvest handling, Processing and Marketing: A Comprehensive Extension Package Manual. Farm Africa, Ethiopia.

French EB, Gutarra I, Aley P, Elphinstone J. 1995. Culture media for *Ralstonia solanacearum* isolation, identification and maintenance. J Phytopathol 30: 26-130.

George F, Sivarana C. 2014. Agronomy of ginger (*Zingiber officinale* Roscoe). J Spice Aromatic Crops 5: 23-45.

Geta E, Kifle A. 2011. Production, processing and marketing of ginger in Southern Ethiopia. J Hortic For 3: 207-213.

Gupta V, Razdan VK. 2013. Evaluation of antagonists and antibiotics against bacterial wilt of brinjal caused by *Ralstonia solanacearum*. J Biol 10 (3): 851-852.

Hayward AC. 1964. Characteristics of *Pseudomonas solanacearum*. J Appl Bacteriol 27: 265-277. DOI: 10.1111/j.1365-2672.1964.tb04912.x.

Hidaka Z, Murano H. 1956. Studies on the streptomycin for plants I. Behavior of *P. solanacearum* and *P. tabaci* treated with streptomycin in vitro and surface absorption of streptomycin in the plant. J Phytopathol 20: 143-147. DOI: 10.3186/jjphytopath.20.143.

Hikichi Y, Yoshimochi T, Tsujimoto S. 2007. Global regulation of pathogenicity mechanism of *Ralstonia solanacearum*. J Plant Biotechnol 24: 149-154. DOI: 10.5511/plantbiotechnology.24.149.

Hunduma T, Sadessa K, Getaneh G. 2016. First report of ginger (*Zingiber officinale*) bacterial wilt disease in Ethiopia. Res J Agric For Sci 4 (4): 5-9.

Indersenan G, Sreekumar V, Mathew J, Mammen MK. 1981. The mode of survival of *Pseudomonas solanacearum* causing bacterial wilt of ginger. J Agric Res 19: 93-95.

Isenegger DA, Taylor PWJ, Mullins K, Mcgregor GR, Barlass M. 2003. Molecular detection of bacterial contaminant *Bacillus pumilusin* symptomless potato plant tissue cultures. Plant Cell Rep 21: 814-820. DOI: 10.1007/s00299-003-0583-z.

Kelman A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. J Phytopathol 44: 693-695.

Khan ANA, Karuna K, Ravikumar MR, Kulkarni RS. 1997. Chemical control of bacterial wilt of tomato caused by to *Ralstonia solanacearum*, the causal agent of bacterial wilt in ginger. Intl J Plant Prot 4 (1): 1-21.

Kumar A, Sarma YR. 2004. Characterization of *Ralstonia solanacearum* causingbacterial wilt of ginger in India. Indian Phytopathol 57: 12-17.

Owoseni AA, Sangoyomi TE. 2014. Effect of solvent extracts of some plants on *Ralstonia solanacearum*. J Microbiol Res 4 (1): 89-96. DOI: 10.9734/BMRJ/2014/4179.

Paul ST. 1998. Biochemical and Biological Basis of Resistance in Solanaceous Vegetables against Bacterial Wilt of *Ralstonia solanacearum* (Smith). [Thesis]. Kerala Agricultural University, Kerala.

Ravindran PN, Babu KN. 2005. Ginger the Ginus *Zingiber*. CRC Press, New York.

Schaad UB, McCracken Jr GH, Loock CA, Thomas ML. 1980. Pharmacokinetics and bacteriological efficacy of moxalactam (LY127935), netilmicin, and ampicillin in experimental gram-negative enteric bacillary meningitis. Antimicrob Agents Chemother 17 (3): 406-411. DOI: 10.1128/AAC.17.3.406.

Schell MA. 2000. Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. Ann Rev Phytopathol 38: 263-292. DOI: 10.1146/annurev.phyto.38.1.263.

Singh DK, Sinha SK, Singh VN, Singh DN. 2000. Control of bacterial wilt of ginger (*Zingiber officinale*) with antibiotics. J Res 12: 41-43.

Singh R, Jagtap GP. 2017. In Vitro evaluation of antibacterial chemicals and bioagents against *Ralstonia solanacearum* infecting bacterial wilt in ginger. Intl J Microbiol 6 (5): 2034-2045. DOI: 10.20546/ijcmas.2017.605.227.

Stromberg KD, Kinkel LL, Leonard KJ. 2004. Quantifying the effect of bacterial antagonists on the relationship between phyllosphere population sizes of *Xanthomonas* translucens pv. translucens and subsequent bacterial leaf streak severity on wheat seedlings. Biol Control 29 (1): 58-65. DOI: 10.1016/S1049-9644(03)00126-9.

Sunder J, Jeyakumar S, Kundu A, Srivastava RC, Kumar DA. 2011. Effect of *Morinda citrifolia* extracts on in-vitro growth of *Ralstonia solanacearum*. Arch Appl Sci Res3 (3): 394-402.

Suslow TV, Schroth MN, lsaka M. 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. J Pathol 72: 917-918. DOI: 10.1094/Phyto-72-917.

Thomas P, Upreti R. 2014. Influence of seedling age on the susceptibility of tomato plants to *Ralstonia solanacearum* during protray screening and at transplanting. Am J Plant Sci 5: 1755-1762. DOI: 10.4236/ajps.2014.512190.

Trujillo EE. 1964. Diseases of Ginger (*Zingiber officinale*) in Hawaii. Hawaii Agricultural Experiment Station, University of Hawaii, Hawaii

Umesha S, Kavitha R, Shetty HS. 2005. Transmission of seed-borne infection of chilli by *Burkholderia solanacearum* and effect of biological seed treatment on disease incidence. J Plant Prot 38 (4): 281-293. DOI: 10.4236/ajps.2014.512190.

Williamson L, Nakaho K, Hudelson B, Allen C. 2002. *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. Plant Dis 86 (9): 987-991. DOI: 10.1094/PDIS.2002.86.9.987.

Winstead NN, Kelman A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. J Phytopathol 42: 628-634.

Zulperi D, Sijama K, Abidin MAZ, Awang Y, Mohd HE. 2014. Phylotype classification of *Ralstonia solanacearum* biovar 1 strains isolated from banana (*Musa* spp.) in Malaysia. Arch Phytopathol 47 (19): 2352-2364. DOI: 10.1080/03235408.2013.876748.