Pak. J. Phytopathol., Vol. 32 (02) 2020. 213-223 DOI: 10.33866/phytopathol.030.02.0596

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

CHARACTERIZATION OF PHYTOPATHOGENETIC STRAINS OF *PSEUDOMONAS* AND *BURKHOLDERIA* AND MANAGEMENT BY BLACK SEED OIL

[a]Mamoona Hassan, [a]Naureen Akhtar, [a]Muhammad Riaz*, [a]Salik N.Khan, [a,b]Muhammad Shakeel, [a]Ateeq Tahir

[a] Institute of Agricultural Sciences, University of the Punjab, Lahore Pakistan.
[b] Agriculture Research Institute (ARI), Sariab-Quetta, Pakistan.

A B S T R A C T

The application of various strategies is being used to manage the Plant diseases. These strategies are used for the management of plant diseases by applying the cultural, genetical, chemical and biological controls. The biological control approaches are getting more importance on account of their less expensive and environmental, friendly usage. Therefore, the research is conducted to evaluate the anti-microbial activity of Nigella sativa seed oil for the management of the most common plant pathogenic gram -ve bacteria pseudomonas and burkholderia. The isolated bacterias are being mentioned here with FCBP reference like *Pseudomonas syringae pv syringae* (FCBP009), P. *Syringae pv populans* (FCBP010), *P. Syringae* (FCBP405), *Burkholderia pseudomallei* (FCBP036; FCBP350; FCBP460) and *B. Glumae* (FCBP459) were collected from infected fruit/seed. The above mentioned bacterial strains were examined by using concentrations (10, 40, 60, 100 and 200 ppm) of Nigella sativa seed oil, ampicillin and streptomycin antibiotics. After investigation, the study found that Nigella sativa maximum inhibition (65.0 mm) were recorded at 200 ppm concentration in assessment of *B. Glumae* (FCBP459) pathogen. Conversely, the minimum inhibition (20.25 mm) were recorded at the similar concentration in assessment of *P. syringae pv. syringae* (FCBP405). Particularly, the present research observes that the antibiotic drugs use against the above mentioned bacteria have shown the great variation in resistance. This research suggests to use of antibacterial materials from biological origin in managing these disease-causing bacteria.

Keywords: Antibiotics, black seed oil, characterization, enzymatic activity, phytopathogens.

INTRODUCTION

Plant development and growth is strongly affected by the existence and activities of microbes. Bacteria can have associative or antagonistic relationships with other microbes moving the composition of the microorganism communities which ultimately affect the growth of plants (Welbaum *et al*., 2004). Bacteria have been extensively studied in association with plant diseases (Francis *et al*., 2010). Chief groups including plant pathogenic bacteria are; Erwinia, Pseudomonas, Ralstonia, Xanthomonas, Agrobacterium, Streptomyces, *Xylella, Arthrobacter, Burkholderia* and Clavibacter. Some crucial phytopathogens include species of *Bacillus, Clostridium, Rhizobacter, Rhizomonas* and *Serratia* (Green *et al.*, 2010; Shenge *et al*., 2007) There is greater diversity in the structure and function of extracellular degradative enzymes of bacteria. These enzymes include; proteases, hemicellulases, xylanases, cellulases and pectinases (Déjean *et al.*, 2013; Lee *et al*., 2014; Toth *et al*., 2003). During infection, bacteria change the physiology of host cells and defense mechanism of the host is suppressed by the pathogen (Rico *et al.*, 2009).

The identification is necessary to cure the diseases or the infection caused due to the bacteria by applying appropriate strategies. Identification of pathogens sustains significance for epidemiological purposes(Ivnitski et al., 1999). Consequently, there is a need to develop fast, precise and sensitive methods for identification of bacteria. Accurate identification facilitates more systematic treatments and disease

Submitted: September 23, 2020
Revised: December 01, 2020
Accepted for Publication: December 02, 2020
* Corresponding Author:
Email: mriaz.iags@gamil.com

prevention (Sharma and Mutharasan, 2013; Tallury *et al*., 2010). Plant extracts are considered to have antibacterial, antiviral, antifungal, insecticidal and antioxidant properties (Burt, 2004; Kordali *et al*., 2005) Several plants and their extracts have been reported for their potential use against microbes including food-borne pathogens (Grujic-Jovanovic et al., 2004; Rančić *et al*., 2005). These essential oils or plant extracts contain a wide variety of plant secondary metabolites that slow or inhibit the microorganism's growth (Nazzaro *et al*., 2013; Stojković *et al*., 2013). Essential oils are mainly composed of mono- and sesquiterpenes which include phenols, alcohols, ethers, carbohydrates, aldehydes and ketones. All these constituents are responsible for biological activity of plant extracts. This is the reason for adding spices and herbs to food since ancient times (Soković *et al*., 2010). Like phenolic and terpenes, other oxygenated terpenoids are shown to have highest antimicrobial activity potential while other hydrocarbons are also potential sources against microbes. Interactions between these types of compounds may lead to antagonistic, additive, or synergistic effects (Bassolé and Juliani, 2012).

In USA, presently, almost 90% of streptomycin is used in plant agriculture to control fire blight (McManus *et al.,* 2002). Minor uses of streptomycin include control of bacterial diseases in floriculture and on potato tubers, tobacco seedlings and other vegetable seedlings in the field or greenhouse (Vidaver, 2002). Ampicillin is also used as an antibacterial drug. It is found effective for various bacterial strains. It is regarded as first 'broad spectrum' pencillin with significant activity against several Gram-positive bacteria including *Streptococcus pyogenes* and *S. pneumoniae*. Gram-negative bacteria i.e. *Haemophilus influenzae* and *Neisseria meningitidis* are also controlled by ampicillin. The combined activity of ampicillin and sulbactem is found to be very effective (Akova, 2008; Hauser, 2012).

Nigella sativa seed also called 'Black Seed' is an ever known miracle seed. This plant has been most extensively studied due to its pharmacological and phytochemical importance. Its aqueous and oil extracts are commonly used because of possessing strong anticancer, anti-inflammatory, anti-analgesic and antimicrobial activities. The most important and abundant component of black seed oil is thymoquinone which is the principal chemical to perform all activities of seed (Gali-Muhtasib *et al*., 2006).

Present study is designed by keeping in view the economic importance of phytopathogenic bacteria and role of authentic identification of pathogens in disease management. For this study, plant pathogenic bacteria were characterized on the basis of morphological and biochemical basis. Oil of *N. sativa* seed was evaluated to determine its efficacy as a biological control agent for these phytopathogens in comparison of commercially available common drugs; ampicillin and streptomycin.

MATERIAL AND METHODS

Procurement of bacterial cultures: For present study, seven plant pathogenic bacterial strains belonging to two different genera isolated from diseased tissues of different plants were selected. Pure cultures of selected bacterial strains were collected from infected fruit/seed and submitted to First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab Lahore for authenticity and got reference accession number. Bacterial strains were stored at 4°C for further study.

Table 1. Inventory of the bacterial strains used for present study.

Strain	FCBP No.	Substrate
Pseudomonas syringae pv. *syringae*	FCBP009	*Prunus savium* fruit
P. syringae pv. *populans*	FCBP010	*Pyrus malus* fruit
P. syringae	FCBP405	*Triticum aestivum* leaf
Burkholderia pseudomallei	FCBP036	*Saccharum officinarum* stem
B. pseudomallei	FCBP350	*Citrus reticulata* fruit
B. pseudomallei	FCBP460	*Oryza sativa* seeds
B. glumae	FCBP459	*Oryza sativa* seeds

Biochemical characterization: Bacteria were revived and pure cultures were subjected to colony description, cell morphological studies and biochemical characterization. Biochemical attributes of selected bacterial pathogens were determined using the bacterial identification system, MicrogenTM GnA+B - ID (Microgen Bioproducts Ltd, UK). The GN-ID identification kit is comprised of two microwell test

strips called GN A and GN B. These microwell strips contain standard substrates required for biochemical analysis. These tests include a preference for carbohydrate as carbon source and activities of enzymes catalyze various metabolic reactions. Identification of the isolates was verified by entering all the biochemical test results into the Microgen Identification System software.

Other biochemical parameters studied during present investigation were cell wall characterization by Gram staining and KOH hydrolysis test; amylase, lipase and arginine dihydrolase enzymes activity tests and finally determination of oxidation/fermentation mode of carbohydrate breakdown by these bacteria.

Cell wall characterization: Cell wall character of each bacterium was determined by standard Gram staining procedure. Potassium hydroxide (KOH) solubility test was also conducted for further analyzing the characteristic of Gram-negative bacteria. A loopful bacterial culture was aseptically removed from Petri plates with tooth pick, placed on a glass slide in a drop of 3% KOH solution. Upon mixing the bacterial cells in KOH, bacteria making mucous thread were regarded as Gram negative while Gram positive bacteria did not form this mucous thread.

Starch hydrolysis test: To test bacteria for this amylase, starch agar medium (Soluble starch 2 g, Peptone 5 g, Beef extract 3 g, Agar 20 g dissolved in 1 L of distilled water) was prepared. Medium was inoculated by streaking bacterial isolates and incubated for 4 days at 27 ± 2°C. After incubation period, plates were flooded with Lugol's iodine solution (Potassium iodide 10 g, Iodine 5 g, distilled water 100 mL) and observed for the appearance of clear zones of hydrolysis around the bacterial growth (Lelliott and Stead, 1987). Amylase activity level is indicated by "+" sign. (+) Positive but poor activity; (++) moderate activity, (+++) denoted to strong activity and (-) is used for no activity.

Lipase activity test: Lipase test, based on the production of lipase enzyme by a bacterium, was also carried out. For lipase enzyme activity, Tween 80 medium was prepared as; Peptone 10 g, NaCl 5 g, $CaCl_2$, H_2O 0.1 g, Agar 15 g, Tween 80 10 mL (1% w/v), distilled water 1 L. Tween 80 agar plates were inoculated with the bacteria and incubated for 7 days at 27 ± 2°C (Lelliott and Stead, 1987).

Arginine dihydrolase test: Thorley's medium (Peptone 1 g, NaCl 5 g, K_2HPO_4 0.3 g, L-arginine HCL 10 g agar 30 g; mixed and dissolved by heating in 1 L distilled water, pH adjusted to 7.2) was used to analyze the arginine dihydrolase ability of selected bacteria. Medium was poured into test tubes at the rate of 5 mL per tube. Medium in the tubes was inoculated with the test bacterial strains by stabbing method. The medium was covered with a layer of sterile mineral oil. Un-inoculated test tube served as control (Lelliott and Stead, 1987). To test the ability of the species to use arginine as carbon source, strains were incubated for 3 days. If the arginine present in medium is utilized, the color of medium changes from orange to pink due to the change in pH of medium.

***In vitro* control of phytopathogenic bacteria**

Selection of antibiotics and plant material: Seed oil of *Nigella sativa* (Kalonji) and two broad-spectrum antibiotics, streptomycin and ampicillin were selected to test their efficacy to control phytopathogenic bacteria.

Preparation of antibacterial materials: One-gram powdered streptomycin or ampicillin was dissolved in 5 mL sterilized deionized water. This stock solution was further diluted in order to check bacterial growth at different doses of antibiotics. Five different concentrations made from the antibiotic stock were 10, 40, 60, 100 and 200 ppm. Sterilized water was used as a control.

Nigella sativa seed oil was used as other tool to control bacterial growth. Concentrated oil purchased from the market was used as stock. From this stock further dilution was prepared by mixing oil with dimethyl sulfoxide (DMSO). Concentrations of *N. sativa* seed oil used to control bacterial growth were 10, 40, 40, 100 and 200 ppm. DMSO was used as control.

Antimicrobial testing setup: To study the antibacterial potential of selected materials, Agar well diffusion method was carried out as described by (Mathabe *et al*., 2006) with some modifications. Bacterial strains were inoculated on agar LBA by spreading 10^3 cells using a sterilized spreader under aseptic conditions. A sterilized cork borer of 5 mm diameter was used to make three wells in each Petri plate at equal distances. Out of the dilutions made from the test samples (seed oil, streptomycin and ampicillin), 50 µl of each treatment was pipetted into the two holes of a plate while one was loaded with control (sterilized water or DMSO). Each treatment was replicated three times.

Bacterial growth condition and result acquisition: Inoculated plates were incubated at 37°C for 24 hours.

Antibacterial effect of selected materials was determined in term of the diameter (mm) of bacterial growth inhibition zone around the wells. Data was presented as mean diameter of inhibition zones of replicates.

RESULTS

Morphological and biochemical characterization: Morphologically, all three strains of *Pseudomonas syringae* were similar with each other. Selected *Burkholderia pseudomallei* strains and *B. glumae* also shared the same colonial as well as cell characteristics (Table 2). However, differences were noted in the morphological characters of *Pseudomonas* and *Burkholderia* strains. Results of biochemical analysis of bacterial cells carried out using MicrogenTM GnA+B – ID system are summarized in Table 2.

Cell wall characterization: Gram's staining results showed that all selected bacteria were Gram negative. In addition, all strains were positive for KOH solubility test hence confirming that these are Gram positive (Figure 1).

Starch hydrolysis by amylase: For the detection of comparative amylase activity, test bacterial strains were grown on starch containing growth medium. After flooding the bacterial colonies with Lugol's iodine solution, of blue/purple color in agar medium indicates the starch. However, if starch of the growth medium is digested by amylase a clear halo zone appears around the colony. Hence bacteria which did not show any hydrolysis zone were regarded as negative for this test (Figure 2). Amylase activity in *Pseudomonas syringae* pv. *syringae* (FCBP009) was found to be moderate however *P. syringae* pv. *populans* (FCBP010) and *P. syringae* (FCBP405) exhibited clear starch hydrolysis zones around colonies, which indicated their excellent amylolytic activity.

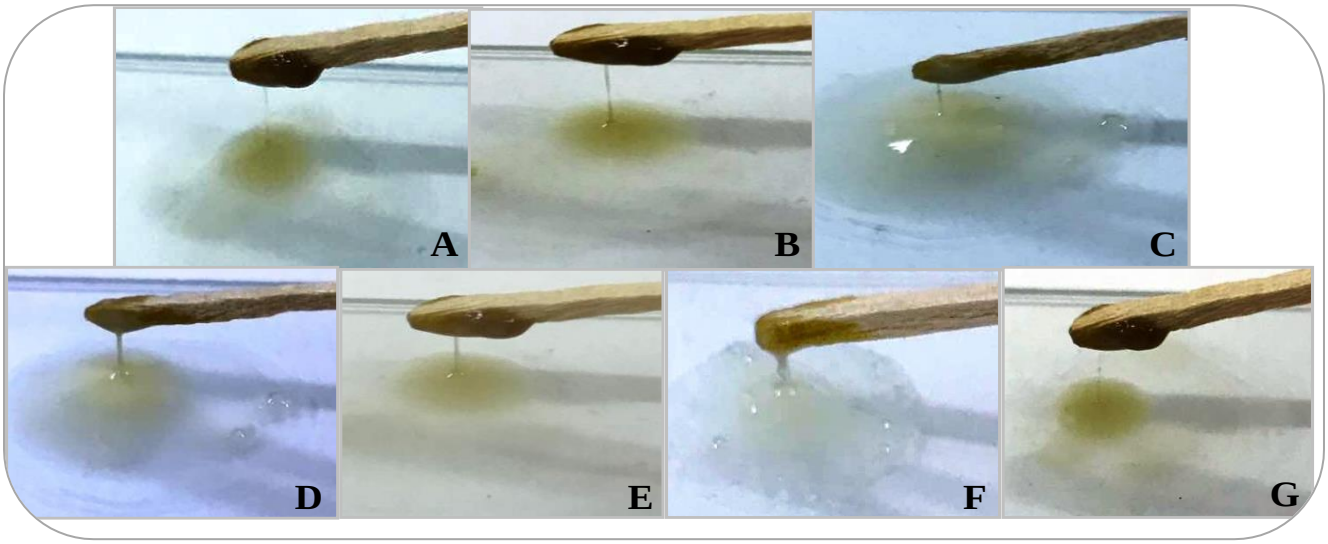

Figure 1. KOH solubility test for cell wall characterization. A: *Pseudomonas syringae* pv. *syringae* (FCBP009); B: *P. syringae* pv. *populans* (FCBP010); C: *P. syringae* (FCBP405); D: *Burkholderia pseudomallei* (FCBP036); E: *B. pseudomallei* (FCBP350); F: *B. pseudomallei* (FCBP460) and G: *B. glumae* (FCBP459).

Table 2. Biochemical characteristics of selected strains as determined by MicrogenTM GnA+B - ID system.

Characters	Name of Bacterial species		
	Pseudomonas syringae (FCBP009, FCBP010, FCBP405)	*Burkholderia pseudomallei* (FCBP036, FCBP350, FCBP460)	*Burkholderia glumae* (FCBP459)
Morphological characters			
Colony color	Off-white	Dirty white	Dirty white
Colony shape	Irregular	irregular	irregular
Surface	Smooth	Rough	rough
Edge	Entire	Entire	entire
Elevation	Raised	Flat	flat
Cell shape	Rods	Rods	rods
Spore type	-ve	-ve	-ve
Capsule stain	-ve	-ve	-ve
Motility test	+ve	-ve	-ve

Biochemical characters			
Indole test	-ve	-ve	+ve
Methyl red test:	-ve	-ve	-ve
Citrate utilization test	-ve	-ve	-ve
Hydrogen sulfide test	-ve	-ve	-ve
Nitrate reduction test	+ve	-ve	-ve
Oxidase test	-ve	-ve	+ve
Catalase test	+ve	-ve	-ve
Gelatin test	-ve	-ve	-ve
Malonate test	-ve	-ve	-ve
Inositol test	-ve	-ve	-ve
Sorbitol test	+ve	+ve	-ve
Rhamnose test	-ve	-ve	-ve
Sucrose test	+ve	-ve	-ve
Lactose test	-ve	-ve	-ve
Arabinose test	-ve	-ve	-ve
Adonitol test	-ve	-ve	-ve
Raffinose test	-ve	-ve	-ve
Salicin test	-ve	-ve	-ve
Mannitol test	-ve	-ve	-ve
Xylose test	-ve	+ve	+ve

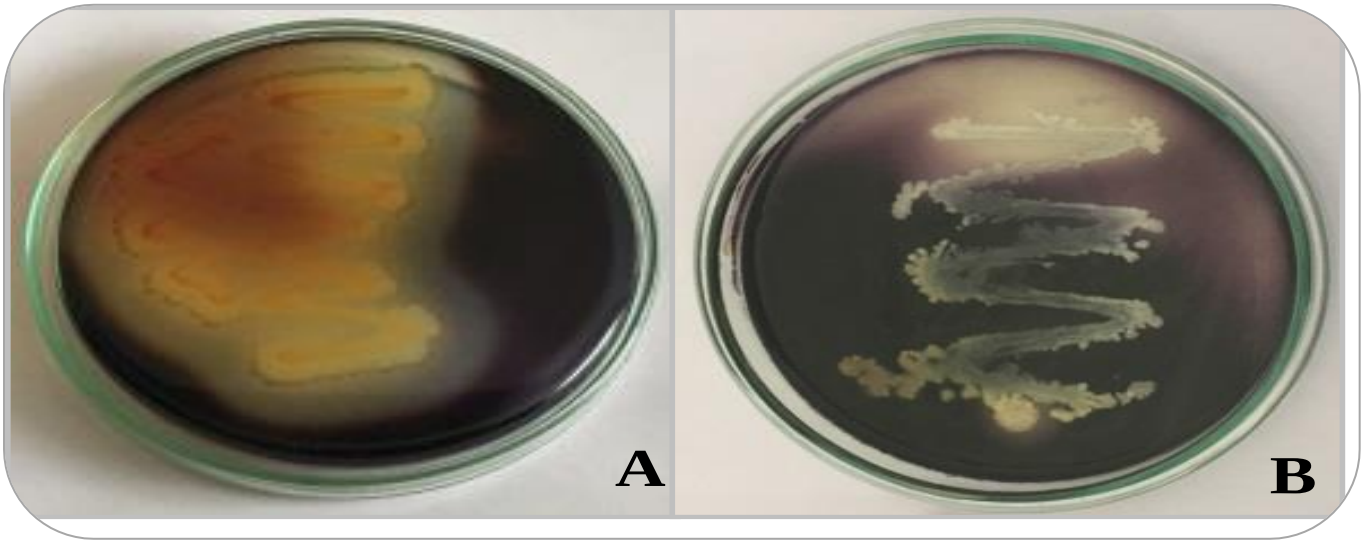

Figure 2. A representation of amylase activity for starch hydrolysis by bacterial strains. A: Strong amylolytic activity; B: No activity.

All selected *Burkholderia* strains viz., *Burkholderia pseudomallei* (FCBP036), *B. pseudomallei* (FCBP350), *B. pseudomallei* (FCBP460) and *B. glumae* (FCBP459) showed strong activity of amylase that could be detected by the clear zone around the growing bacterial colonies.

Lipase activity test: The lipase activity of bacterial cells was determined in term of the presence of white precipitation around the bacterial colonies when grown on Tween 80 agar plates (Figure 3). *Pseudomonas syringae* pv. *syringae* (FCBP009) showed positive result for lipase activity while *P. syringae* pv. *populans* (FCBP010) and *P. syringae* (FCBP405) did not show precipitation around the colonies indicating that both of these strains were devoid of lipase activity.

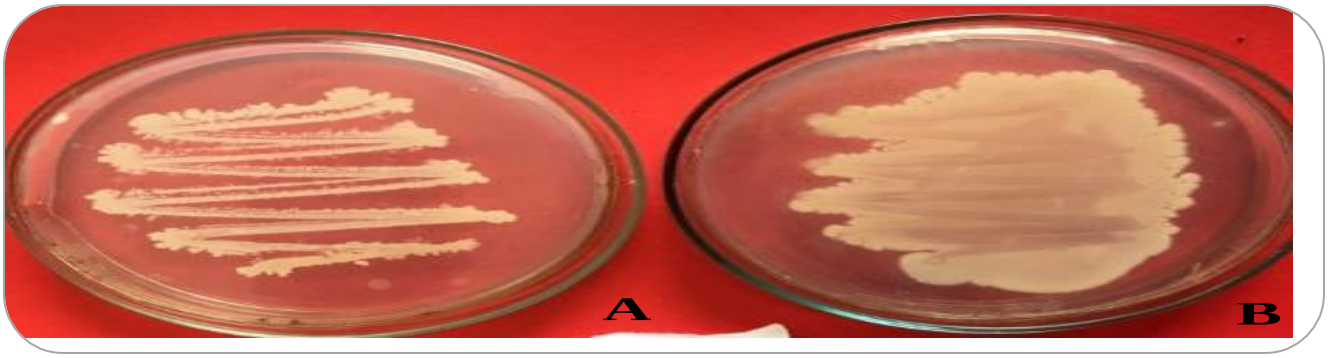

Figure 3. Lipase activity of a bacterial pathogen. A: No substrate added; B: Substrate added.A dense white precipitation due to lipase activity was observed around the colonies of *B. pseudomallei* (FCBP036) and *B. pseudomallei* (FCBP460) depicting strong lipase activity by these stains. On the other hand, *B. pseudomallei* (FCBP350) and *B. glumae* (FCBP459) showed moderate activity.

Arginine dihydrolase test: Results of present study showed that one strain of *Pseudomonas* i.e. *P. syringae* pv. *syringae* (FCBP009) exhibited pink color indicated its positive activity for arginine hydrolase (Figure 4) while other two strains; *P. syringae pv. populans* (FCBP010) and *P. syringae* (FCBP405) showed negative results. All the selected *Burkholderia* strains were unable to utilize arginine present in growth medium.

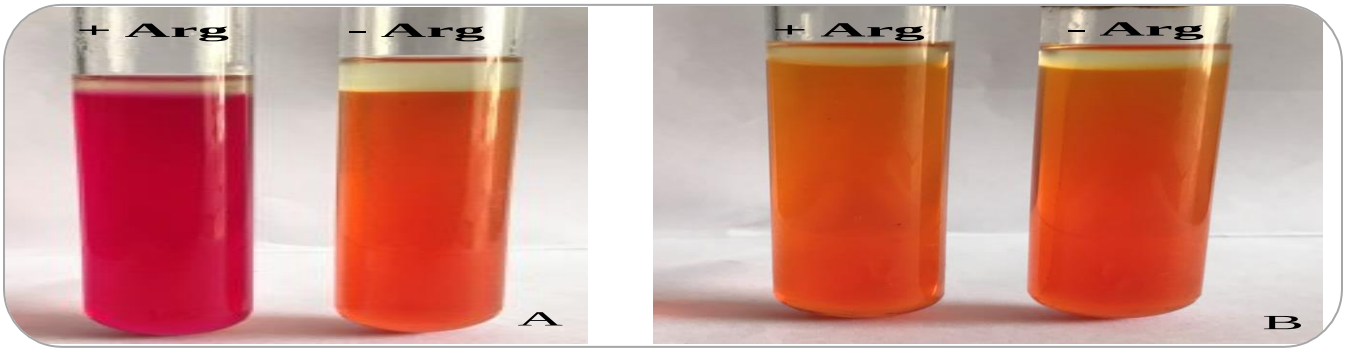

Figure 4. A representative demonstration of arginine dihydrolase test results. A: Arginine dihydrolase activity positive and B: Arginine dihydrolase activity negative. + Arg denoted to arginine added to medium while – Arg means arginine is absent in medium.

Management of bacterial pathogens: Seed oil of *Nigella sativa* was tested for its efficacy in controlling the growth of bacterial pathogens. The antibacterial potential of *N. sativa* seed oil was compared with two commercially available broad-spectrum antibiotics; Ampicillin and streptomycin. A range of different concentrations of selected antibacterial compounds was evaluated and their effect was measured in term of the diameter of bacterial growth inhibition zone (Figure 5).

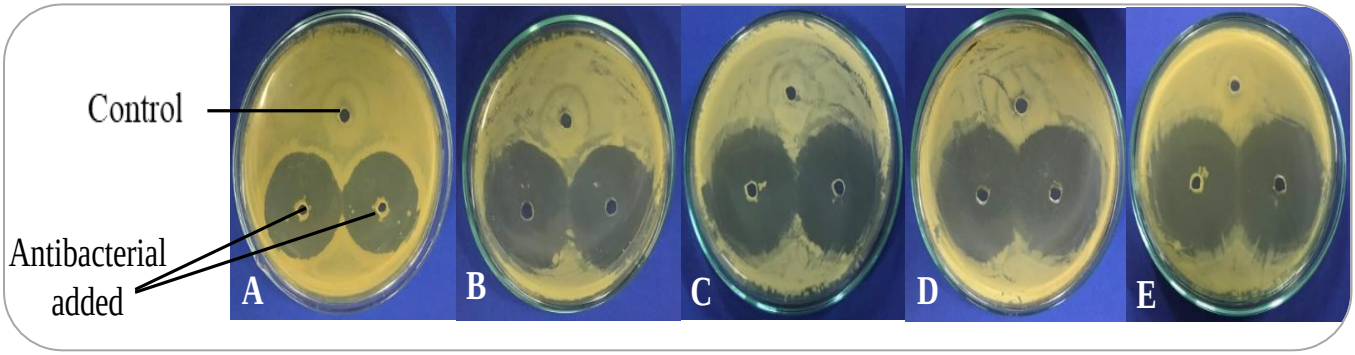

Figure 5. Experimental set up to study antibacterial effect of selected antibiotics/black seed oil. Well on top of each plate is showing control (without antibacterial compound). Other two wells in plate are replicates for each treatment. A: 10 ppm; B: 40 ppm; C: 60 ppm; D: 100 ppm and E: 200 ppm antibacterial added.

Growth inhibition of *Pseudomonas* species: In general, an increase in diameter of inhibition zone was recorded as the concentration of black seed oil increased from 10 to 200 ppm hence highest concentration presented strongest antibacterial effect on growth of all three *Psuedomonas* strains. Antibacterial potential of oil was comparable with that of commercially available antibiotics (Figure 6) against all the tested strains. Surprisingly, black seed oil caused more growth inhibition than the ampicillin in case of *P. syringae* pv. *syringae* (FCBP009) and *P. syringae* pv. *populans* (FCBP010). However black seed oil when compared to commercial antibiotics, had least effect on growth of *P. syringae* pv. *syringae* (FCBP405). All concentration of oil showed almost equal values for growth inhibition zones of *P. syringae* pv. *syringae* (FCBP405). Comparing effect of all three antibacterial compounds, streptomycin posed highest growth control in all tested bacteria (Table 3).

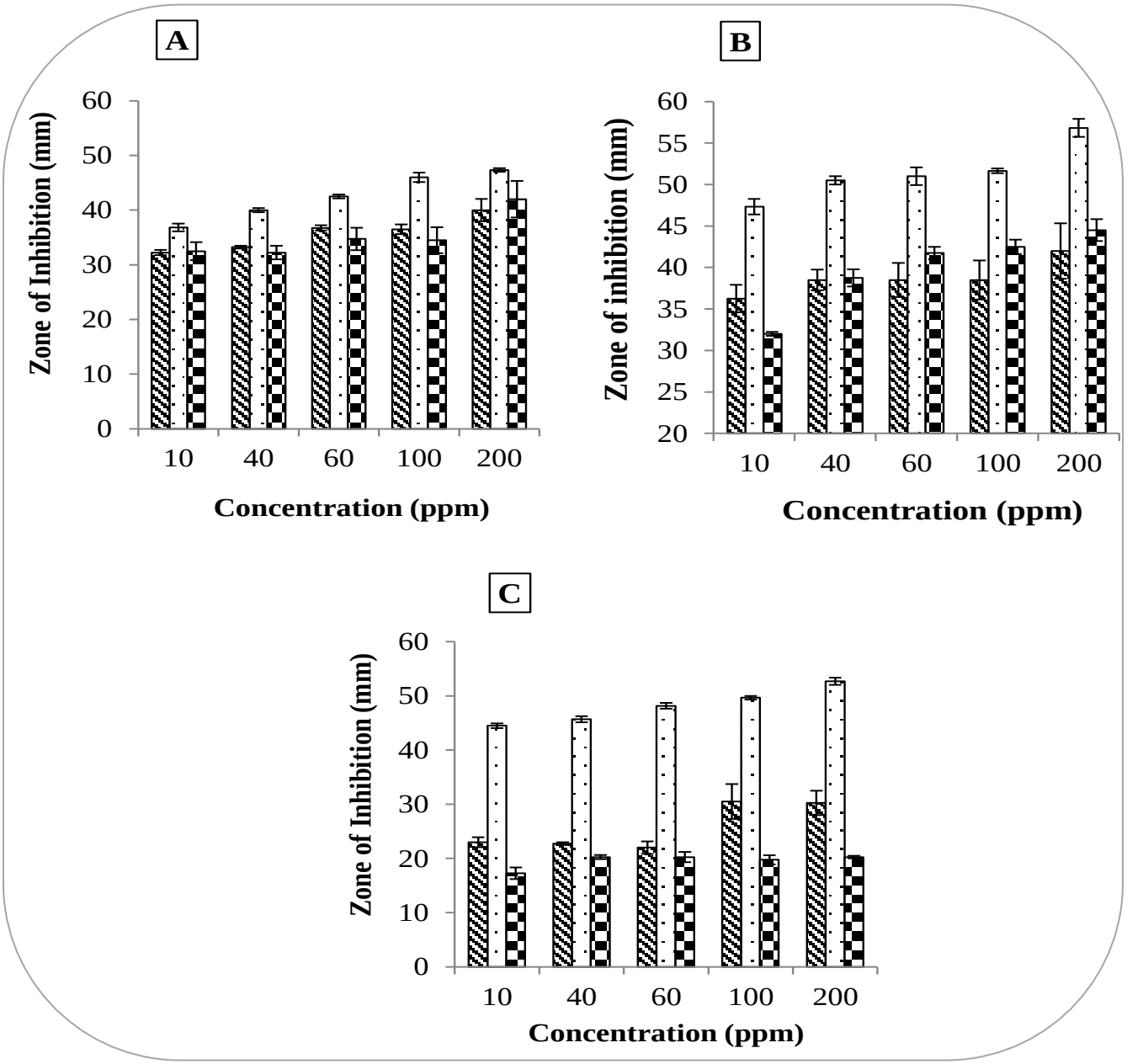

Figure 6. Comparative antibacterial potential of ampicillin (▨), streptomycin (▤) and black seed oil (▚) on growth of A: *Pseudomonas syringae* pv. *syringae* (FCBP009*)*; B: *P. syringae* pv. *populans* (FCBP010) and C: *P. syringae* pv. *syringae* (FCBP405). Vertical bars represent the standard error (SE) of the mean of replicates of each treatment.

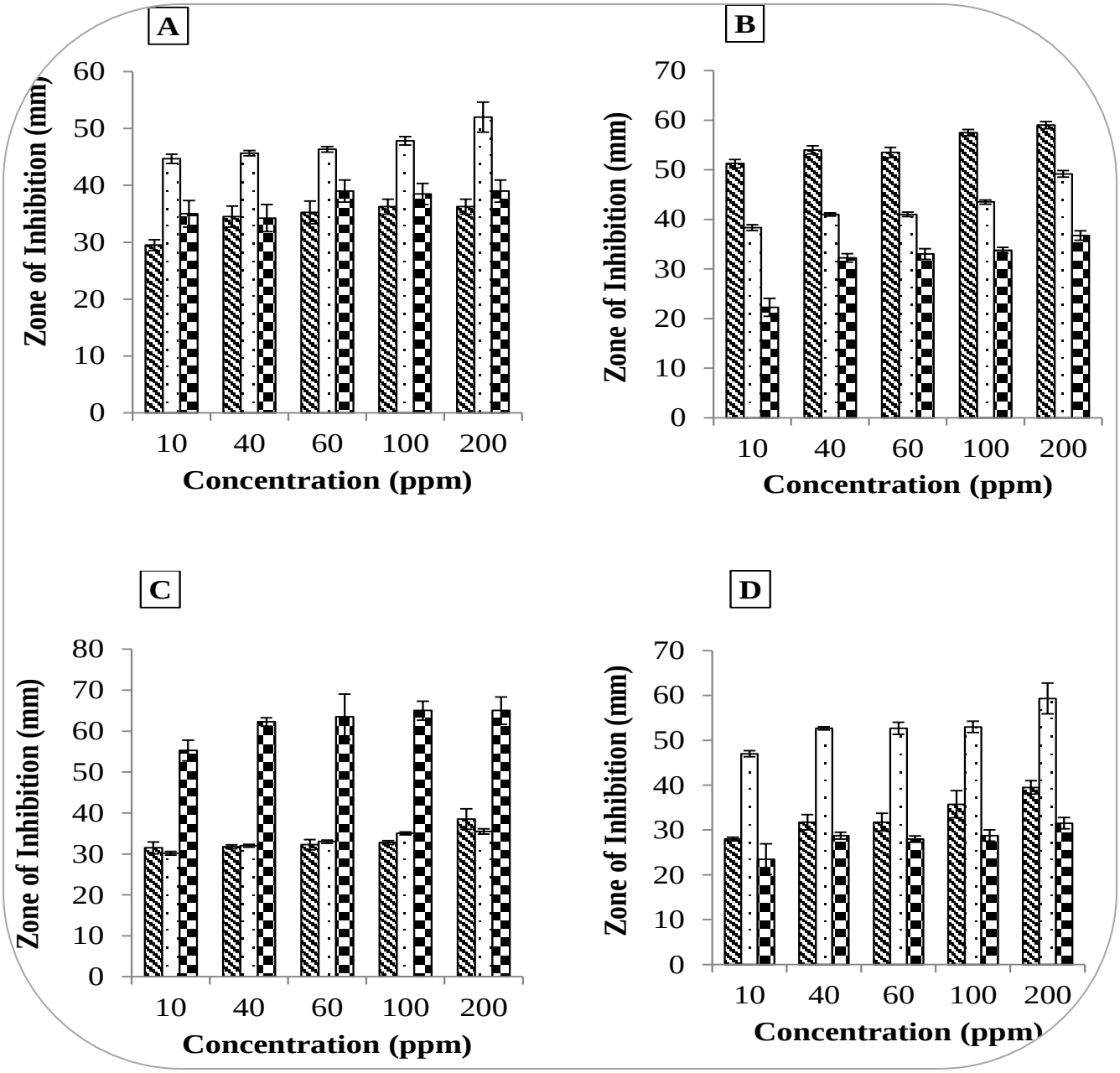

Figure 7. Comparative antibacterial potential of ampicillin (▨), streptomycin (▤) and black seed oil (▩) on growth of A: *Burkholderia pseudomallei* (FCBP036); B: *B. pseudomallei* (FCBP350); C: *B. glumae* (FCBP459) and D: *B. pseudomallei* (FCBP460). Vertical bars represent the standard error (SE) of the mean of replicates of each treatment.

Table 3. Diameters of growth inhibition zones when applied maximum (200 ppm) concentration of antibacterial compound

Strains	Black seed oil	Ampicillin	Streptomycin
P. syringae pv. *syringae* (FCBP009)	42.15 + 3.34	40.20 + 2.04	47.33 + 0.33
P. syringae pv. *populans* (FCBP010)	44.50 + 1.32	42.20 + 1.08	56.83 + 1.51
P. syringae (FCBP405)	20.25 + 0.25	30.25 + 2.25	52.67 + 0.67
B. pseudomallei (FCBP036)	39.10 + 1.96	36.25 + 1.31	52.05 + 2.62
B. pseudomallei (FCBP350)	36.75 + 0.95	59.05 + 0.71	49.17 + 0.67
B. pseudomallei (FCBP460)	31.50 + 1.26	39.55 + 1.5	59.33 + 3.45
B. glumae (FCBP459)	65.10 + 3.34	38.55 + 2.5	35.10 + 0.65

Growth inhibition studies of *Burkholderia* strains: Following concentrations of black seed oil, different levels of bacterial growth checks were recorded for *Burkholderia* species. Order of inhibition was observed as *B. glumae* (FCBP459) > *B. pseudomallei* (FCBP036) > *B. pseudomallei* (FCBP350) > *B. pseudomallei* (FCBP460). Black seed oil was found to be highly effective against *B. glumae* (FCBP459) growth as significant increase in growth inhibition zone was observed by increasing concentration. In response to black seed oil, *B. glumae* (FCBP459) zone of inhibition diameter was almost double as that by ampicillin and streptomycin (Table 3). Growth of *B. pseudomallei* (FCBP460) was maximum controlled by streptomycin followed by ampicillin while black seed oil exhibited least antibacterial potential (Figure 7). Black seed oil exhibited higher antibacterial potential against *B. pseudomallei* (FCBP036) than ampicillin while in comparison to commercial drugs, proved to be less effective in controlling growth of *B. pseudomallei* (FCBP350).

DISCUSSION

Current investigation was attempted to characterize the phytopathogenic bacteria on biochemical basis and analyze their comparative sensitivity towards streptomycin, ampicillin and oil of black seeds. Gram-negative bacteria have been found highly associated with plant diseases. Moreover, their association with respect to plant diseases has been extensively studied and established (Francis et al., 2010). All strains studied for present study were Gram negative and also formed a mucoid loop to further confirm cell wall characterization. (Jabeen et al., 2012) performed same experiment with different isolates of *Xanthomonas oryzae* pv. *oryzae*, provided all strains were Gram negative and also showed positive KOH solubility results. With respect to other enzymatically catalyzed reactions, working on the parallel lines, (Gormez et al., 2013) performed similar biochemical tests for characterization of different isolations of *Psuedomonas syringae* and came with different results for different strains. Similarly, (Prakash et al., 2014) did similar experiment on isolation, identification and characterization of *Burkholderia pseudomallei* from soil. They isolated 67 bacterial strains from soil and performed their biochemical test to check their similarities. All tested strains provided positives results for arginine and glucose test while only eight isolates were found biochemically consistent to profile of *B. pseudomallei* except with negative arabinose sugar assimilation.

For present study, antibacterial efficacy of black seed oil was compared with that of two very commonly used commercial antibiotics i-e streptomycin and ampicillin. Synthetic antibiotics target bacterial gyrase and cause cell death (Dwyer et al., 2007). Streptomycin mainly affects bacterial ribosomes and influenced protein biosynthesis (Kaji and Kaji, 1965). In present study, different concentrations of ampicillin and streptomycin tried were 10, 40, 60, 100 and 200 ppm. All of these caused different effects on bacterial growth. However, a trend was observed with these concentrations that the lowest concentration had the least effect on bacterial growth while the highest concentration had a remarkable effect on bacterial growth. Similar work was performed by Ingham and Coleman, 1986) who found that low streptomycin concentration did not reduce bacterial population, while high concentration applications showed good effect.

Similar work was also conducted to find antibacterial activity by (Sebiomo et al., 2011) to investigate the effect of ginger (*Zingiber officinale*) extract and some commercially available antibiotics i.e. chloramphenicol, ampicillin and tetracycline against *Streptococcus pyogenes* and *S. aureus*. Ginger extracts were found very effective against these bacteria while different concentrations of three antibiotics showed significant effect on the inhibition zones of both bacteria. The *In vitro* activity of gentamicin, tobramycin, kanamycin, and amikacin in combination with ampicillin was determined against aminoglycoside-resistant group *B streptococci*. Synergy in each combination was determined by quantitative kill curves and demonstrated in all the combinations tested (Cooper et al., 1979).

Nigella sativa seeds were found effective for fungal, parasitic and bacterial infections. For centuries, its oil is used as good analgesic, food preservative, condiment and carminative and for treatment of many ailments around the world (Dadgar et al., 2006). Ether extract and its derivatives like thymoquinone play a major role for antimicrobial activity by *N. sativa*. These chemicals were found very effective to inhibit *Scopulariopsis brevicaulis*, *Fusarium solani* and *Aspergillus niger* which are known as opportunistic fungi. Several species of three genera of dermatophytes i.e. *Microsporum*, *Trichophyton* and *Epidemophyton* are found to control by *N. sativa* (Aljabre et al., 2015).

(Topozada, 1965) first reported that phenolic fraction of *N. sativa* had antibacterial effects. In an experiment, black seed extract and essential oil were tested for their antioxidant, antifungal and antibacterial activities. Both of these extracts were found effective against *Penicillium citrinum*, while in case of bacteria, there was complete inhibition of growth zones of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilus* and *B. cereus* with strong antioxidant activities (Singh et al., 2005).

Present investigation suggested that different bacterial strains even belonging to same species behave differentially towards their enzymatic activity and oil seed *N. sativa* has the potential to control the plant pathogenic bacteria.

REFERENCES

Akova,M. 2008.Sulbactam-containing β-lactamase inhibitor combinations. Clinical Microbiology and Infection, 14: 185-188.

Al-Ghamdi, M. 2001. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Journal of Ethnopharmacology, 76: 45-48.

Aljabre, S. H., O. M. Alakloby and M. A. Randhawa. 2015. Dermatological effects of *Nigella sativa*. Journal of Dermatology & Dermatologic Surgery, 19: 92-98.

Bassolé, I. H. N. and H. R. Juliani. 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17: 3989-4006.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods a review. International Journal of Food Microbiology, 94: 223-253.

Coleman, D. 1986. The role of microfloral and faunal interactions in affecting soil processes, Microfloral and faunal interactions in natural and agro-ecosystems. Springer, 3:317-348.

Cooper, M., R. Keeney, S. Lyons and E. Cheatle. 1979. Synergistic effects of ampicillin-aminoglycoside combinations on group *B streptococci*. Antimicrobial agents and Chemotherapy, 15: 484-486.

Dadgar, T., M. Asmar, A. Saifi, M. Mazandarani, H. Bayat, A. Moradi, M. Bazueri and E. Ghaemi. 2006. Antibacterial activity of certain Iranian medicinal plants against *methicillin*-resistant and sensitive *Staphylococcus aureus.* Asian Journal of Plant Sciences, 5: 861-866.

Déjean, G., S. Blanvillain-Baufumé, A. Boulanger, A. Darrasse, T. D. de Bernonville, A. L. Girard, S. Carrére, S. Jamet, C. Zischek and M. Lautier. 2013. The xylan utilization system of the plant pathogen *Xanthomonas campestris pv campestris* controls epiphytic life and reveals common features with oligotrophic bacteria and animal gut symbionts. New Phytologist, 198: 899-915.

Dwyer, D. J., M. A. Kohanski, B. Hayete and J. J. Collins. 2007. Gyrase inhibitors induce an oxidative damage cellular death pathway in *Escherichia coli*. Molecular Systems Biology, 3: 91.

Francis, V. A., A. Zorzano and A. A. Teleman. 2010. dDOR is an EcR coactivator that forms a feed-forward loop connecting insulin and ecdysone signaling. Current Biology, 20: 1799-1808.

Gali-Muhtasib, H., N. El-Najjar and R. Schneider-Stock. 2006. The medicinal potential of black seed (*Nigella sativa*) and its components. Advances in Phytomedicine, 2: 133-153.

Gormez, A., F. Sahin, M. Gulluce and I. Aslan. 2013. Identification and characterization of *Pseudomonas syringae* isolated from apricot trees in the erzurum province of Turkey and evaluation of cultivar reaction. Journal of Plant Pathology, 3: 525-532.

Green, S., D. J. Studholme, B. E. Laue, F. Dorati, H. Lovell, D. Arnold, J. E. Cottrell, S. Bridgett, M. Blaxter and E. Huitema. 2010. Comparative genome analysis provides insights into the evolution and adaptation of *Pseudomonas syringae pv. aesculi* on *Aesculus hippocastanum.* Public Library of Science, 5: 1020-10224.

Grujic-Jovanovic, S., H. D. Skaltsa, P. Marin and M. Sokovic. 2004. Composition and antibacterial activity of the essential oil of six Stachys species from Serbia. Flavour and Fragrance Journal, 19: 139-144.

Ivnitski, D., I. Abdel-Hamid, P. Atanasov and E. Wilkins. 1999. Biosensors for detection of pathogenic bacteria. Biosensors and Bioelectronics, 14: 599-624.

Jabeen, R.,T. Iftikhar and H. Batool. 2012. Isolation, characterization, preservation, and pathogenicity test of *Xanthomonas oryzae pv. oryzae* causing BLB disease in rice. Pakistan J. Bot, 44: 261-265.

Kaji, H. and A. Kaji. 1965. Specific binding of sRNA to ribosomes: effect of streptomycin. Proceedings of the National Academy of Sciences of the United States of America, 54: 213.

Kordali, S., R. Kotan, A. Mavi, A. Cakir, A. Ala and A. Yildirim. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera* essential oils. Journal of Agricultural and

Food Chemistry, 53: 9452-9458.

Lee, D. H., J.-B. Kim, J.-A. Lim, S.-W. Han and S. Heu. 2014. Genetic diversity of *Pectobacterium carotovorum subsp. brasiliensis* isolated in Korea. The plant pathology journal, 30: 117.

Lelliott, R. A. and D. E. Stead. 1987. Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, p216.

Mathabe, M., R. Nikolova, N. Lall and N. Nyazema. 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. Journal of Ethnopharmacology, 105: 286-293.

McManus, P. S., V. O. Stockwell, G. W. Sundin and A. L. Jones. 2002. Antibiotic use in plant agriculture. Annual review of phytopathology, 40: 443-465.

Nazzaro, F., F. Fratianni, L. De Martino, R. Coppola and V. De Feo. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6: 1451-1474.

Prakash, A., D. Thavaselvam, A. Kumar, A. Kumar, S. Arora, S. Tiwari, A. Barua and K. Sathyaseelan. 2014. Isolation, identification, and characterization of *Burkholderia pseudomallei* from soil of coastal region of India. Springerplus, 3: 438.

Rančić, A., M. Soković, J. Vukojević, A. Simić, P. Marin, S. Duletić-Laušević and D. Djoković. 2005. Chemical composition and antimicrobial activities of essential oils of *Myrrhis odorata (L.)* Scop, Hypericum perforatum L and Helichrysum arenarium (L.) Moench. Journal of Essential Oil Research, 17: 341-345.

Rico, A., R. Jones and G. M. Preston. 2009. Adaptation to the plant apoplast by plant pathogenic bacteria. Plant pathogenic bacteria: genomics and molecular biology: 63-89.

Sebiomo, A., A. Awofodu, A. Awosanya, F. Awotona and A. Ajayi. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale)* on two pathogenic bacteria. Journal of Microbiology and Antimicrobials, 3: 18-22.

Sharma, H. and R. Mutharasan. 2013. Review of biosensors for foodborne pathogens and toxins. Sensors and actuators B: Chemical, 183: 535-549.

Shenge, K., R. Mabagala, C. Mortensen, D. Stephan and K. Wydra. 2007. First report of bacterial speck of tomato caused by *Pseudomonas syringae pv*. tomato in Tanzania. Plant disease, 91: 462-462.

Singh, G., P. Marimuthu, C. S. de Heluani and C. Catalan. 2005. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds. Journal of the Science of Food and Agriculture, 85: 2297-2306.

Soković, M., J. Glamočlija, P. D. Marin, D. Brkić and L. J. Van Griensven. 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules, 15: 7532-7546.

Stojković, D., J. Glamočlija, A. Ćirić, M. Nikolić, M. Ristić, J. Šiljegović and M. Soković. 2013. Investigation on antibacterial synergism of *Origanum vulgare* and *Thymus* vulgaris essential oils. Archives of Biological Sciences, 65: 639-643

Tallury, P., A. Malhotra, L. M. Byrne and S. Santra. 2010. Nanobioimaging and sensing of infectious diseases. Advanced drug delivery reviews, 62:424-437.

Toth, I. K., K. S. Bell, M. C. Holeva and P. R. Birch. 2003. Soft rot *erwiniae*: from genes to genomes. Molecular Plant Pathology, 4:17-30.

Vidaver, A. K. 2002. Uses of antimicrobials in plant agriculture. Clinical infectious diseases, 34: 107-110.

Welbaum, G. E., A. V. Sturz, Z. Dong and J. Nowak. 2004. Managing soil microorganisms to improve productivity of agro-ecosystems. Critical Reviews in Plant Sciences, 23: 175-193.

Contribution of Authors :

Mamoona Hassan	:	Conceptualized the study. Formal analysis, methodology and writing of original manuscript
Naureen Akhtar	:	Reviewed and edited the manuscript.
Muhammad Riaz	:	Review and editing and technical input in writing manuscript
Salik N. Khan	:	Provided technical guidelines during the study
Muhammad Shakeel	:	Compilation and interpretation of data
Ateeq Tahir	:	Field visits for collection of data and Figures and Graphs in Microsoft excel