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Evaluation of Essential Oils for *in-vitro* Management of *Dickeya dadantii* Causing Soft Rot of Onion

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Onion is known as one of the most vital grown vegetable crops which in addition to its quality as a vegetable is being used as salad also in all households, a variety of factors (biotic/abiotic) affects it. Onion is susceptible to various diseases and it is being affected by post-harvest diseases such as bacterial soft rot, it is a decaying bacteria, that causes heavy losses to the onion and also affects the quality by deteriorating the onion and causing heavy economic losses. The most harmful vegetable ailment is regarded as soft rot. It may be found everywhere there are fleshy storage tissues of onion. Five treatments of distinct essential oils were prepared using various concentrations of 5, 10 and 15%, and treatment six was kept as a control. All the concentrations were prepared and were applied against *Dickeya dadantii* causative agent of soft rot of onions on nutrient agar inoculating plates for their efficacy. The essential oils showed better antibacterial activities against the pathogen *Dickeya dadantii* in all three concentrations 5%, 10%, 15%. soft rot control (21.33%) was recorded in the case of peppermint oil which is considered as highest, after that by cinnamon oil (17.74%), followed by Kalvanji oil (11.74%), and other oils like Lemon oil and Sesame oil recorded (8.74%) and (3.74%) respectively. However, there was found no inhibition zone in control that was kept without applying any essential oil. The data was statistically analyzed.

Keywords: Onion, *Dickeya dadantii*, characterization, essential oils, *in-vitro*, disease management, antibacterial activity.

INTRODUCTION

Onions (Allium cepa L.) are known as an important vegetable crop being grown and consumed in Pakistan and throughout the world (Griffiths et al., 2002). The Amaryllidaceae family includes Allium cepa from the Latin language, in which cepa means "onion"), often known as the bulb onion or common onion (Chase et al., 2009). All around the world, onions are grown and utilized. They can be consumed as raw food or used to produce different foodstuffs like pickles and chutneys etc., nevertheless, onions are frequently presented cooked, either as a vegetable or as a part of a savory dish, when chopped, and they have a strong flavor and contain certain chemicals that might irritate the eyes (Block et al., 2010). Various color onion bulbs including White, yellow, or red color are edible to be consumed. It contributes in the diet of humans with low calories and has medicinal value with low fat (Slimestad et al., 2007). Onion has medicinal qualities by decreasing the cholesterol level in the blood of humans alternatively chance of clots in the vessels is reduced and improves blood circulation. It is helpful in diabetes treatment also by lowering the sugar level in the blood and minimizing arteriosclerosis (Kumar et al., 2010). Developing countries lack the proper storage facility alternatively disease (soft rot of onion) development chances increase because of high temperature (25-30°C) (Bdliya & Haruna, 2007). As compared to other bacterial diseases, bacterial soft rot heavily effects the Post-harvest onions (Agrios, 2007). During post-harvest handling bruising or often insects create wounds in the plants which provides the primary source for the bacterium to enter in plant tissues and after that disease starts the development in favorable summer conditions because of favorable temperatures (Higashio & Yamada, 2004). However, disease management is one of the important

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strategies; so various methods may be adopted which may include agricultural practices like balanced fertilizers and other strategies including timely harvesting and post-harvest curing and treatment; such practices are helpful to control bacterial soft rot of onion bulbs and prevent the spread of the disease in storage condition (Abdalla et al., 2013). The onion is a crop that requires little preservation, although significant degradation might still happen during storage as a result of sprouting, physiological weight loss, and decaying. Storage losses may amount to 66% or more (Biswas et al., 2010). *D. dadantii* causing bacterial soft rot has its place in the family Pectobacteriaceae, the pathogen is a gram-negative bacillus, known as *Erwinia chrysanthemi* (Samson et al., 2005). Several host plants are affected by soft rot diseases brought on by the phytopathogenic bacterium *D. dadantii*, including those that are commercially significant (Grenier et al., 2006). Early signs include lesions covered in water at the infection site, and chlorotic tissue loss of turgor (Komatsu et al., 2002). The anticancer effects of orange and peppermint oils have been proven (Kumar et al., 2004). Antioxidant properties can be found in lemon oil & rosemary (Calabrese et al., 1999). It has been proven that most essential oils contain effective properties as not only antibacterial but also antiviral, insecticidal, and antioxidant. For usage in alternative medicine to treat bacterial and fungal infections, many essential oils have been suggested (Okla et al., 2019). It has been proven that cinnamon oil, clove oil, and rosemary have effective quality to suppress bacteria and fungi and cinnamon oil has antidiabetic possessions too. According to reports, basil contains anti-inflammatory effects (Babu et al., 2007).

MATERIALS AND METHODS

***Samples collection*:** Diseased onion bulb samples were collected from Quetta market based on visibly *known typical* symptoms; which include water-soaking soft rot or yellowish brown color rot on onion and characteristic fragrance as described by (Shing, 1985) and were brought into the laboratory of the plant pathology department, Baluchistan agriculture college, Quetta.

***Isolation and purification of samples*:** The infected onion pieces were cut into small (2-3 mm) pieces after being thoroughly washed in sterile water & dipped in sodium hypochlorite 2% for surface sterilization. These sterilized pieces were then placed in petri plates with Nutrient Agar medium and incubated for 2 to 5 days to allow the pathogen to fully develop. Restreaking of bacterial colony was done for purification as performed by Schaad (2001).

***Pathogenicity test*:** A pathogenicity test was performed in which healthy bulbs of onion were used. For inoculation of pathogen wounded onions were prepared for which sterile toothpicks were used. For obtaining the bacterial inoculum two days old cultures were used and maintained on KB broth. Incubation of onion bulbs was done in a moist chamber for 7 days. These were examined daily until symptoms developed on onion bulbs. Re-isolating was performed from decaying and browning onion bulbs with the same symptoms on *KB medium* (Naqqash et al., 2016).

***Characterization and identification of bacterial isolates*:** For characterization of the pathogenic isolates of *Dickeya Dadantii* Causing Soft Rot Of Onion; a Series of tests physiological and biochemical were performed in which gram staining was performed as prescribed by Coico (2006). Growth at 37°C (Ostlie et al., 2005). Salt tolerance & pH Tolerance (Pandey & Singh, 2012) were followed. The Yeast Dextrose Calcium Carbonate (YDCC) test was performed (Emitaro, 2016). Performed the Amylase (starch hydrolysis) test (Padma & Pallavi, 2016). Potassium hydroxide (KOH) Solubility test (Mahesh et al., 2017). Catalase production test as prescribed by Reiner (2010).

***In vitro application of Essential oils*:** Five essential oils were used at the laboratory Plant pathology of Baluchistan Agriculture College Quetta. Essential oils Viz., Peppermint oil, cinnamon oil, kalvanji oil, lemon oil, and sesame oil with more than 98% pure in quality were selected. For screening disk diffusion methods were used to check the efficient essential oil as prescribed by Burt (2004). 10 ml of Mueller Hinton Broth was used for culture isolation and the culture was 18 hours old which were maintained at 37°C. it was adjusted with sterile saline solution (105CFU/ml approximate). Suspension of 500ml were feasted over the Mueller-Hinton agar plate with the help of a cotton swab and maintained uniformity for further growth of microbes on test plates as well as control plates. All the procedures of dissolving of essential oils, adding of these in distilled water and preparation of NA, and pouring in plates were done, Tween 20 (0.1%v\v) was used for its dissolving, these plates were stored at the appropriate temperature and three concentrations (5, 10 & 15%) were prepared for testing, all the procedures were performed in aseptic condition (Wayne, 2002). In the control plates ethanol & tween 20 with similar quantities were used and mixed in the NA media. Sterile Para film was used for wrapping the plate so that evaporation or contamination to be avoided in the test sample. Only diffusion of oil was allowed by keeping the plates at room temperature for up to 30 minutes after that incubated as captioned above for the development of inhibition zones and data was collected.

***Statistical Analysis*:** The statistical analysis was done by one-way analysis of variance (ANOVA) to check the means followed by Tukey's multiple comparison test. For these analyses, Statistix 8.1 software was used.

RESULTS AND DISCUSSION

***Morphological characterization of bacteria*:** The visual and microscopic observation of the samples which were placed on plates having NA medium was done after 24 hours of incubation at 27°C. To identify the bacterial colonies morphology on plates. The colony colors of every isolate were detected as light brown. The size of the colonies was found intermediate to the lesser, rod in shape & irregular form with uniform undulate boundaries and raised elevation (Table 1 supplementary data).

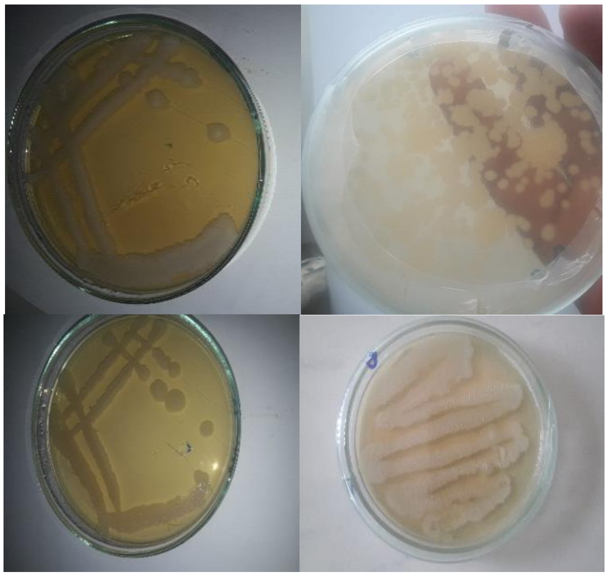

Figure 1. Isolation of bacteria spread as light brown colonies around blighted sample plated on NA plate. B, streaking of bacteria for obtaining pure culture.

***Biochemical characterization*:**Several biochemical tests were conducted to characterize the isolates of the pathogen, the result of all isolates Figure 2.

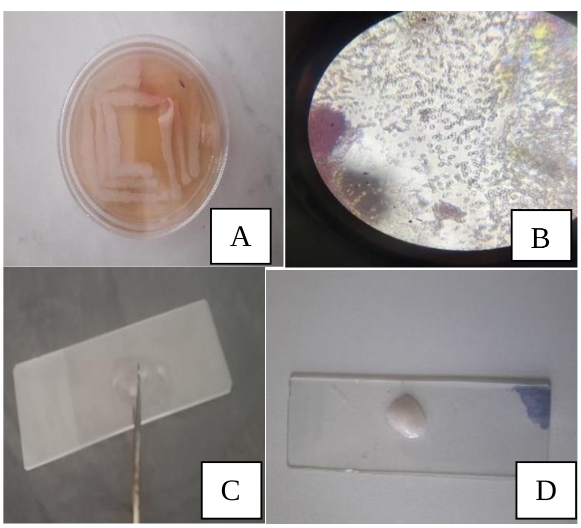

Figure 2. (A) bacterial Culture (B) Microscopic view (C) Biochemical test (d) slide for test.

Pink color bacterial cells were observed in Biochemical tests analysis of the bacterial isolates after Gram's staining, Growth at 39°C, NaCl 3.0%, pH 8.5, Yeast Dextrose Calcium Carbonate (YDC) test, Starch hydrolysis (Amylase) test, Potassium Hydroxide (KOH 3%), Catalase production test. The pathogen was a Gram-negative bacteria and its shape was a rod (*bacillus*) with a speckled arrangement. Bacterial smearing exposed as Pink color. Under control conditions at 37°C showed positive growth. Salt (NaCl) tested in 1.0%, 3.0%, and 5.0%, solution showed Positive growth. pH test in 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, solution showed positive growth. Yeast Dextrose Calcium Carbonate (YDC) added into the ager showed no yellow colonies so found negative. Soft rot bacteria have not used starch in the important Amylase test (Starch hydrolysis test) and it has become Negative bacteria. Another test of Potassium hydroxide (KOH 3%) in this viscous suspension produced strands and showed negative results. Gas bubble were shown by the soft rot bacteria in Catalase test which confirms its positive result.

Table 1. Biochemical tests were performed against soft rot bacteria isolated from onion.

Sr.	Test Names	Results
1.	Gram staining	Gram-negative (-)
2.	Growth at temperature 37°C	Growth at 37°C Positive (+)
3.	Salt & pH tolerance	NaCl and pH result Positive (+)
4.	YDC Test	YDC result Negative (-)
5.	Amylase test (starch hydrolysis test)	Amylase Negative (-)
6.	KOH test (3%)	KOH result Positive (+)
7.	Catalase test	Catalase positive (+)

Pathogenicity test: The pathogenicity test was performed by using infected and healthy onions. Onions were first disinfected with 1 percent NaCl solution for 3 minutes and dried. Then the portion of diseased parts was transferred into blotter paper in petri dishes. Then NA was prepared and the sample was cut for 3 mm diameter using the cork borer from the growing point of the culture. The cultures were then incubated at 12h in light and 12h in dark conditions. Similarly, that culture was inoculated and kept under incubation for one week. Isolation from the onion, samples showed prominent symptoms (Naqqash et al., 2016) which resulted in the same symptoms by inoculated onion and control onion with no symptoms.

***In vitro management through Essential oils*:** The results of the anti-bacterial action of essential oils viz., Peppermint oil, cinnamon oil, kalvanji oil, lemon oil, and sesame oil against soft-rot bacteria, the results of the experiment show that the essential oils had antibacterial activities with unpredictable magnitudes. The control Petri plate results showed a zone of inhibition above 7 mm in diameter.

Figure 3. After inoculation onion shows the typical symptoms of leaf spot.

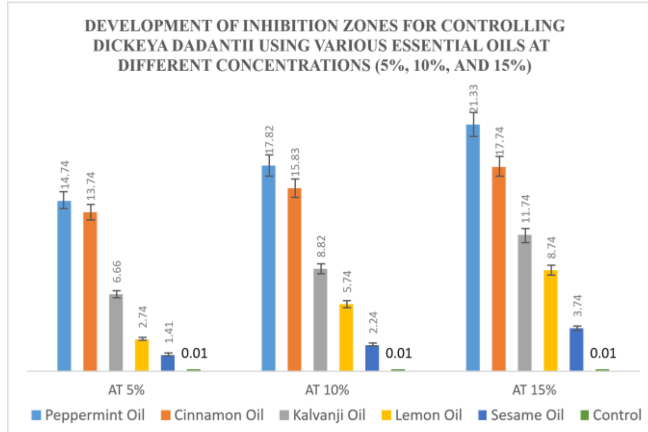

Figure 4. Development of inhibition zones for controlling *Dickeya dadantii* using various essential oils at different concentrations (5%, 10%, and 15%).

Generally, the isolated bacteria were found sensitive to several essential oils used in the experiment. Five treatments showed an antibacterial effect against *Dickeya dadantii*. Nevertheless, the difference was found between essential oils. This research stated that the occurrence of causative plant pathogenic bacteria of soft rot of onion so far verifies the activity of some essential oils that show effects against the growth of *Dickeya dadantii* colonies in vitro. Food is preserved naturally through essential oils. The pharmaceutical industry also uses them for their antimicrobial activity. Essential oils registered all over the world are herbal products that prevent or inhibit the growth of bacteria (Prabuseenivasan, 2006). The study showed that the effect of essential oils treatments at effective concentration was used to control the disease incidence, severity and its weight loss of postharvest onion caused by soft rot of bacteria over untreated. Peppermint oil showed the most effective control in minimizing the disease incidence & followed by cinnamon oil. (21.33%) was recorded by peppermint oil, after that cinnamon oil (17.74%) showed effective results, Kalvanji oil (11.74%), Lemon oil (8.74%), and Sesame oil (3.74%). Sesame oil (1.41%) result showed the lowest effect against *D. dadantii spp*. after 4 days of data and has a lesser effect on the percent disease severity of the pathogen. All the treatments showed significant effects to minimize the pathogen as compare to the control. Peppermint at 15% concentration was found effective oil against the pathogen. But there was found no inhibition zone in control that was kept without applying any essential oil. The study shows that peppermint oil is the most effective antimicrobial agent against the isolated pathogen Gram negative bacteria as compare to other all treatments and control plate were found without inhibition zones.

***Development of inhibition zone at various concentrations through various oils*:** In an overall comparison test of essential oils reaction between various essential oils and their effect on bacterial colony growth on agar plates in vitro condition. Totally six treatments were used in order to show their efficacy in which one of them was kept as control where essential oils were not applied. The essential oils showed better antibacterial activities against the pathogen *Dickeya dadantii* in all three concentrations 5%, 10%, and 15%. High efficacy was shown at 15% concentration as compared to lower concentrations. Essential oil peppermint oil was best with efficacy (21.33%) followed by cinnamon oil (17.74%), kalvanji oil (11.74%), lemon oil (8.74%), sesame oil (3.74%) were less effective as compared with other essential oils. Peppermint oil was excellent in the reduction of bacterial colony growth when applied at concentrations 5%, 10%, and 15% respectively. But there is no inhibition zone was found in the control that was without any essentials.

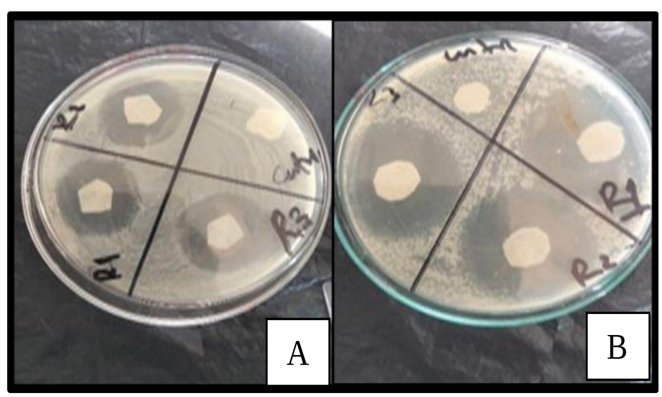

Figure 2. (A) Inhibition Zone and control (B) highest Inhibition Zone and control.

***Conclusion*:** It was concluded based on present study that the maximum inhibition zone was observed at 15% concentration treated with peppermint oil against *Dickeya dadantii*. It will be more advantageable to emphasize the management of *D. dadantii* with herbal plant essential oil (peppermint oil). Further research is required on a large scale in different areas to check its efficacy.

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***Ethical statement*:** No ethics committee permission was required

***Availability of data and material*:** All data is published in this article.

***Consent for publication*:** All authors submitted consent to publish this article in Phytopathogenomics and Disease Control.

***SDGs addressed*:** Zero Hunger, Responsible Consumption, and Production.

***Policy referred*:** Integrated Pest and Disease Management (IPM) Policy; Post-Harvest Loss Reduction Policy; Sustainable Agriculture and Organic Farming Promotion; national and international agricultural policies.

REFERENCES

Agrios, G.N., (2007). Bacterial soft rots. In: G.N. Agrios (ed.), Plant pathology, Fifth edition. Elsevier Academic Press, San Diego, USA; p.656.

Abdallah, M.E., (2013). Application of actinomycetes as biocontrol agents in the management of onion bacterial rot diseases. *Archives of Phytopathology and Plant Protection 46*, 1797-1808.

Babu, P.S., (2007). Cinnamaldehyde a potential antidiabetic agent. *Phytomedicine 14*, 15-22.

Bdliya, B.S., & Haruna H.U., (2007). Efficacy of solar heat in the control of bacterial soft rot of potato tubers caused by *Erwinia carotovora* ssp. *carotovora*. *Journal of Plant Protection Research 47*, 11-18.

Biswas, S.K., (2010). Yield and storability of onion (*Allium cepa* L.) as affected by varying levels of irrigation. Bangladesh *Journal of Agricultural Research 35*, 247-255.

Block, E., (2010). Applications of direct analysis in real time mass spectrometry (DART-MS) in Allium chemistry. 2-propenesulfenic and 2-propenesulfinic acids, diallyl trisulfane S-oxide, and other reactive sulfur compounds from crushed garlic and other Alliums. *Journal of Agricultural and Food Chemistry 58*, 4617-4625.

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

Chase, M.W., (2009). A subfamilial classification for the expanded Asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society 161*, 132-136.

Calabrese, V., (1999). Biochemical studies on a novel antioxidant from lemon oil and its biotechnological application in cosmetic dermatology. *Drugs under Experimental and Clinical Research 25*, 219-225.

Coico, R., (2006). Gram staining. *Current Protocols in Microbiology 1*, A-3C doi.org/10.1002/ 9780471729259.mca03cs00

Emitaro, W.O., (2016). Morphological, biochemical and molecular characterization of *Xanthomonas campestris* pv. vesicatoria of African nightshades (*Solanum scabrum* Mill.). *International Journal of Biosciences 11*, 190-197.

Griffiths, G., (2002). Onions; a global benefit to health. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 16*, 603-615.

Grenier, A.M., (2006). The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. *Applied and Environmental Microbiology 72*, 1956-1965.

Higashio, H., (2004). Control of soft rot after harvest of cabbage in Indonesia. *Japan Agricultural Research Quarterly 38*, 175-178.

Komatsu, T., (2002). Bacterial wilt of China aster caused by *Erwinia chrysanthemi*. *Journal of General Plant Pathology 68*, 105-107.

Kumar, K.S., (2010). *Allium cepa*: A traditional medicinal herb and its health benefits. *Journal of Chemical and Pharmaceutical Research 2*, 283-291.

Kumar, A., (2004). Anticancer and radioprotective potentials of *Mentha piperita*. *Biofactors 22*, 87-92.

Mahesh, M.S., (2017). Potassium hydroxide solubility test to determine protein quality of soybean meal. *Indian Journal of Animal Nutrition 34*, 118-120.

Naqqash, T., (2016). Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. *Frontiers in Plant Science 7*, 144. doi.org/10.3389/fpls.2016.00144

Okla, M.K., (2019). Yield, phytochemical constituents, and antibacterial activity of essential oils from the leaves/twigs, branches, branch wood, and branch bark of Sour Orange (*Citrus aurantium* L.). *Processes 7*, 363. doi.org/10.3390/pr7060363

Ostlie, H.M., (2005). Effect of temperature on growth and metabolism of probiotic bacteria in milk. *International Dairy Journal 15*, 989-997.

Padma, S., (2016). Isolation and characterization of amylase producing *Bacillus* spp. from selected soil sample. *International Journal of Research in Biosciences 5*, 24-29.

Pandey, S., (2012). Organic solvent tolerance of an α-amylase from haloalkaliphilic bacteria as a function of pH, temperature, and salt concentrations. *Applied Biochemistry and Biotechnology 166*, 1747-1757.

Prabuseenivasan, S., (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine 6*, 1-8.

Reiner, K., 2010. Catalase test protocol. *American society for microbiology*, pp.1-6.

Sarkar, M.J.A., (2012). Pathogenicity of the bacterial isolate *Aeromonas hydrophila* to catfishes, carps and perch. *Journal of the Bangladesh Agricultural University 10*, 157-161.

Samson, R., (2005). Transfer of *Pectobacterium chrysanthemi* and *Brenneria paradisiaca* to the genus. *International Journal of Systematic and Evolutionary Microbiology 55*, 1415-1427.

Schaad, N.W., (2001). Laboratory guide for the identification of plant pathogenic bacteria (No. Ed. 3). *American Phytopathological Society 50*, 812-814.

Shing, R.S., (1985). Diseases of Vegetable Crops. Oxford and IBA Publishing Company Pvt. Ltd. New Delhi; p.331.

Slimestad, R., (2007). Onions: a source of unique dietary flavonoids. *Journal of Agricultural and Food Chemistry 55*, 10067-10080.

Wayne, P.A., (2002). NCCLS (National Committee for Clinical Laboratory Standards) Method for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. *Approved Standard*, pp. M100-S12.