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IDENTIFICATION OF THE CAUSAL AGENT OF BACTERIAL SOFT ROT OF CHILLIES AND ITS MANAGEMENT

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Abstract

It's no doubt that chilli peppers are a lucrative cash crop for farmers all around the globe. Heavy crop losses were incurred as a result of the many variables that affected chilli crop productivity, such as the weather, insect pests, soil quality, and a lack of good producing cultivars. However, abiotic and biotic elements pose the most significant risk to the chilli crops, while biotic factors account for significant annual losses. The damage caused by bacteria is greater than that caused by any other kind of infection. Infectious soft rot of chillies, a significant issue caused by Pectobacterium carotovorum, poses a considerable challenge. Through biochemical and physiological testing, the pathogen responsible for soft rot in chillies was identified and declared as Pectobacterium carotovorum spp. caratovora. To assess the pathogenicity, a mechanical inoculation approach was employed in conducting tests on healthy chillies. Moreover, the study explores the management of pathogen through essential oil (Neem oil, Mint oil, Lemon oil and Cinnamon oil) were prepared by using varied concentrations of 5%, 10%, and 15% and treatment of fifth kept as control. All the concentrations were prepared and were applied against Pectobacterium carotovorum causative agent of soft rot of chillies on nutrient agar inoculating plates for their efficacy. Mint showed highest value of 18.33% inhibition zone, while Lemon found less effective with minimum inhibition zone of 1.79%. Although, the study was in-vitro but it can be very helpful for better management in-vivo condition as well.

Keywords: *Chillies, Essential oils, Pectobacterium carotovorum*

INTRODUCTION

Chilli, scientifically classified as *Capsicum annum* L. and *Capsicum frutescens* L., commonly known as red pepper, belongs to the *Solanaceae* family (1). In American English, it is referred to as 'Bell Pepper' or 'Chili Pepper,' while in British English; all species of Capsicum are collectively labeled as 'Peppers.' Notably, in India and Australian English, the term 'Capsicum' is specifically reserved for non-pungent varieties (2). The genus Capsicum consists of approximately 31 different species, out of which five have gained worldwide cultivation significance. These include *C. annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens*. The other species in this genus are predominantly found in the wild (3). The production of chilli crops is subject to a multitude of influences, encompassing environmental factors, insect pests, diseases, water scarcity, soil fertility, and a shortage of high-yielding varieties. These collective factors have been identified as significant contributors to substantial losses in chilli crops (4).Various pathogens, including fungi, bacteria, viruses, and nematodes, are responsible for causing diseases in chilli crops. Notably, bacterial soft rot stands out as a globally significant barrier, inflicting considerable damage to chilli yields (5). The detrimental soft rot

disease, attributed to *Pectobacterium carotovorum (Syn. Erwinia carotovora),* poses a threat to numerous economically vital vegetables, including Carrot, Cabbage, Cucumber, Eggplant, Garlic, Onion, Pepper, Potato, Radish, Sweet Potato, Squash and Tomato (6). *Erwinia* soft rot stands out as a significant and perilous disease affecting vegetables, with a global distribution wherever fleshy storage tissues of vegetables and ornamentals are present. This bacterial affliction leads to more substantial overall losses in produce compared to other bacterial diseases. Its impact is pervasive, affecting crops in the field, during transit, in storage, and even during the marketing phase, resulting in considerable economic setbacks (7). The utilization of chemical bactericides to manage soft rot bacteria encounters resistance due to their non-persistence, associated side effects, elevated costs, and the potential development of resistance within bacterial populations (8). Essential oils have an important role in nature by protecting plants and acting as antifungal, antibacterial, antiviral, and insecticidal agents and also protection against herbivores by reducing appetite of herbivores for plants with such properties (9). It is proven that efficacy of certain botanicals ginger, turmeric, neem, coriander, garlic, lemon against bacterial soft rot of tomato in vitro was tested at different concentration (10).

MATERIALS AND METHODS

SAMPLE COLLECTION

The samples of chilli fruit displaying characteristic symptoms of soft rot were gathered from Jabalnoor, Killi Almas, and the local market in the Quetta. Subsequently, these samples were transported to the Plant Disease Analytical Lab, Department of Plant Pathology, Balochistan Agriculture College, Quetta, for further analysis.

ISOLATION OF THE *PECTOBACTERIUM CAROTOVORA*

The infected plant tissues of chili peppers, along with healthy portions, were carefully separated under a laminar flow hood. The samples were then subjected to sterilization using a 1% sodium hypochlorite solution (NaOCl) for 60 seconds, followed by thorough rinsing with distilled water. These sterilized tissue pieces were aseptically placed in Petri plates containing nutrient agar (NA) medium. The Petri plates were incubated at 27°C for 2-3 days and monitored daily for bacterial growth.

PATHOGENICITY TEST

A pathogenicity test was conducted using healthy chilli peppers. The chilli peppers were disinfected with a 1% sodium hypochlorite solution (NaOCl) solution for 3 minutes then washed with distilled water and allowed to dry. Isolated bacteria were inoculated in nutrient broth for pathogenic test followed by 24 h incubation and bacterial suspension was prepared at a concentration of 108 CFU/ml. The solutions were injected into fruits of host plants (chillies) placed in polyethylene bags with wet papers and these bags were maintained under incubation for one week (11).

CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

A series of biochemical and physiological tests were performed for identification of the pathogen as described by Akbar *et al.,* 2015 (12).

IN-VITRO EVALUATION OF ESSENTIAL OILS

Four essential oils, namely neem oil, lemon oil, peppermint oil, and cinnamon oil, were sourced from a certified scientific firm for chemicals and lab equipment in Pakistan. These oils were selected based on a comprehensive literature review and their established applications in conventional medicine. Their quality was determined to exceed 98% purity. For the initial screening, a bacterial zone of inhibition and disc diffusion method, following the approach by (13) with slight modifications, was employed. A 24-hour young bacterial culture was prepared to achieve a concentration of approximately 105 CFU/ml. Uniform microbial growth was ensured on both control and test plates using a sterile cotton swab. The essential oils

were dissolved by blending them with a mixture of ethyl alcohol, distilled water (C_2H_5OH), and tween 20 (0.1 v/v). Three different concentrations, namely 5%, 10%, and 15%, were examined. Each treatment consisted of three Petri plates, each containing one impregnated paper disc placed at the center. As a control, paper discs soaked in sterile distilled water were used. The Petri plates were then incubated at 27°C for 90 hours. After the incubation period, the zone of inhibition surrounding the filter paper disc was measured to assess the effectiveness of the various oils (14).

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERIZATION OF BACTERIA

The samples placed on NA plates showed the bacterial colonies after 24 hours of incubation at 27 C temperatures. The visual and microscopic observation was done to identify the colony morphology. The colony color of all isolates exhibited creamy, somewhat shiny light-colored colonies with medium to small sizes. These colonies displayed a rod-shaped morphology with straight borders (15).

BIOCHEMICAL TEST

After 3 days, the chilli samples were observed for rottening. All samples were showing the developed symptoms of soft rot. Our results are similar as described by Abu-Obeid, 2019 (7).

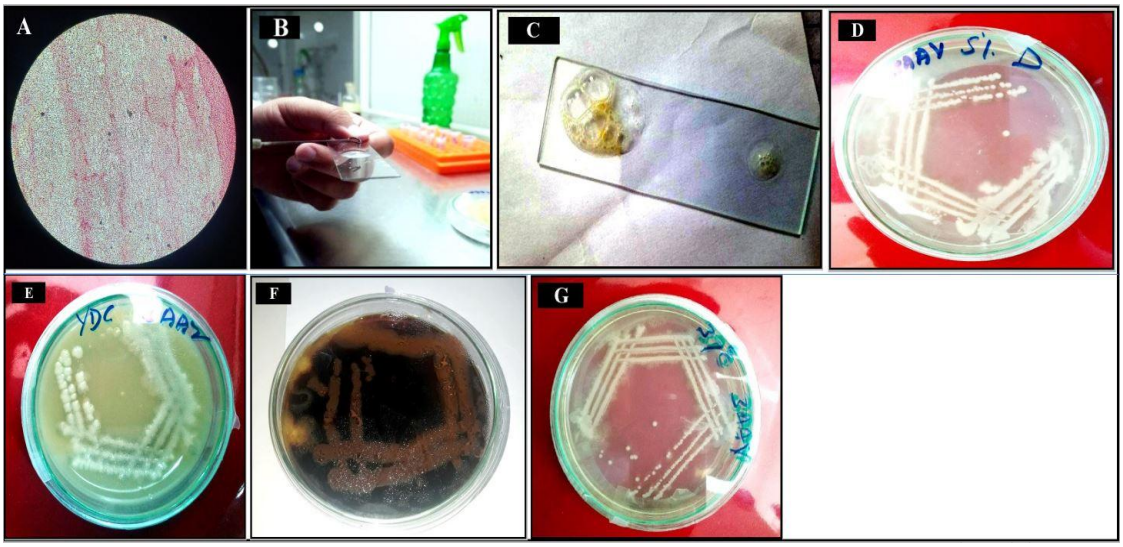

Fig. 1. Biochemical test; (A) Gram`s Staining (B) KOH (C) Catalase test (D) NaCl 5% (E) YDC test (F) pH 0.8 (G) Growth at 37°C

The result of gram staining of all isolates was gram negative and these results are similar with the result of Nazerian (16).The result of Catalase test all isolates were showing positive result and KOH test of all isolates were negative these result are according to Akbar (12). The result of amylase **(**Starch Hydrolysis) test of all isolates were negative these result are according to Akar *et al.,* 2019 (13).The result of salt tolerance of all isolates was positive and these results were alike to the result of Akbar *et al.,* 2015 (12), While the result of Yeast Dextrose Calcium Carbonate (YDC) test of all isolates were negative and these results were according to the results Mikhail (15).

DEVELOPMENT OF INHIBITION ZONE FOR CONTROL AT 5% CONCENTRATION USING DIFFERENT ESSENTIAL OILS

According to the results, *Pectobacteriumcarotovorum* was controlby essential oils for various treatments, Peppermint, Neem, Lemon and Cinamon oils all had noticeable results. Peppermint oil at a 5% concentration after control treatment displayed a maximal inhibition zone with a value of 13.24% over the pathogen. Lemon oil at a concentration of 5% after control treatment had a minimal inhibitory zone with a value of (1.79) over the pathogen.

Table I. The biochemical test result of different isolates

Isolates number	Isolation points	Names of Tests						
		Gram staining	Catalase test	KOH (3%)	Salt Tolerance	Growth at 37˚C	Amylase test (Starch hydrolysis test)	YDC (Yeast Dextrose Calcium Carbonate)
1	Jabal noor	-	+	-	+	+	-	-
2	Jabal noor	-	+	-	+	+	-	-
3	Jabal noor	-	+	-	+	+	-	-
4	Killi Almas	-	+	-	+	+	-	-
5	Killi Almas	-	+	-	+	+	-	-
6	Local Market	-	+	-	+	+	-	-
7	Local Market	-	+	-	+	+	-	-
8	Local Market	-	+	-	+	+	-	-
9	Local Market	-	+	-	+	+	-	-
10	Local Market	-	+	-	+	+	-	-

The maximum amount was found in 5% concentration of peppermint oil of effectiveness against the pathogen *Pectobacteriumcarotovorum*, with a value of (13.24mm). While the essential oils at 5% concentration in the control group did not provide a statistically significant result. Lemon oil demonstrated a minimal inhibitory zone with a value of (1.79mm) over the pathogen as compared to the control treatment. Our finding is similar with Kara (13).

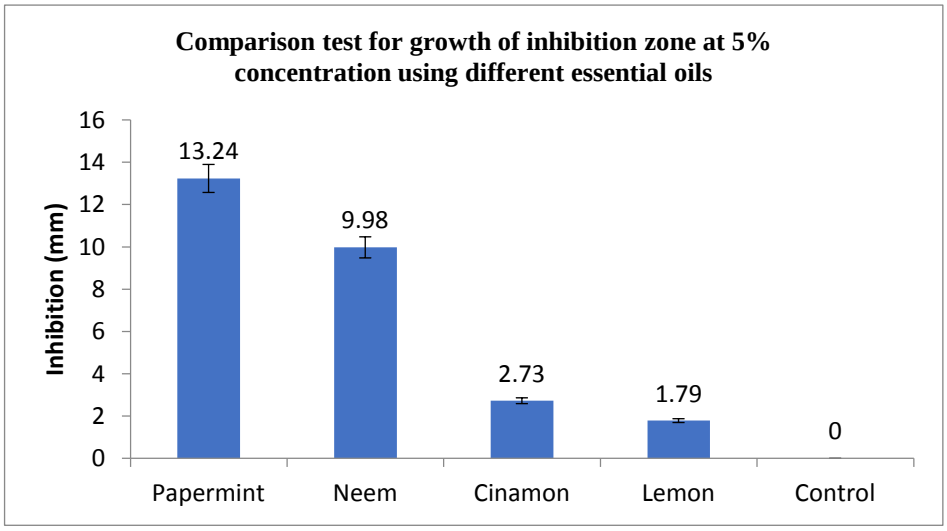

Fig. 2. Comparison test for growth of inhibition zone at 5% concentration using different essential oils

DEVELOPMENT OF INHIBITION ZONE FOR*PECTOBACTERIUMCAROTOVORUM* CONTROL OF AT 10% CONCENTRATION USING DIFFERENT ESSENTIAL OILS

The findings indicate that the pathogen *Pectobacteriumcarotovorum* was most effectively controlled by peppermint oil at a 10% concentration (15.91). While in the case of essential oils, control is nonexistent. Therapy with essential oils on several days all had noticeable results, including therapy with Neem oil, lemon oil, peppermint oil and Cinamon oil. Lemon oil at a 10% concentration after receiving control treatment had the smallest inhibitory zone with a value of (2.24) over the pathogen.

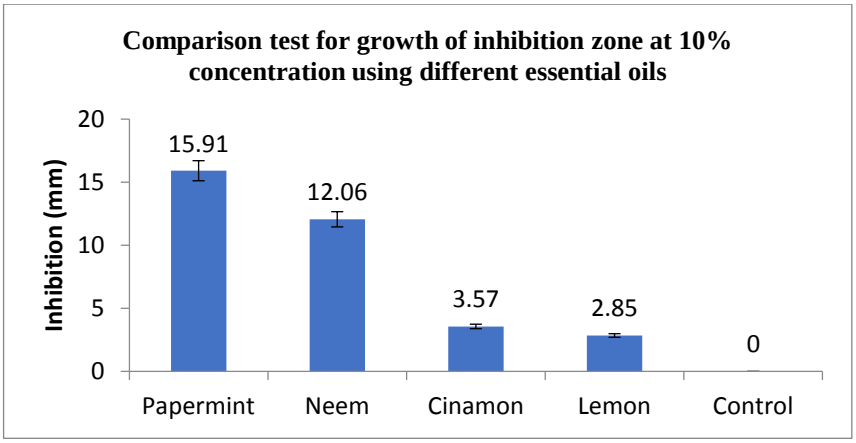

Fig. 3. Comparison test for growth of inhibition zone at 10% concentration using different essential oils

According to the results the pathogen *Pectobacteriumcarotovorum* was most effectively controlled by peppermint oil at a 10% concentration (15.91). The essential oils at a 10% concentration in the control group, however, produced non-significant results. Lemon oil demonstrated a minimal inhibitory zone with a value of (2.865) over the pathogen as compared to the control treatment. Our finding is similar with El-habbak & Refaat (17).

DEVELOPMENT OF INHIBITION ZONE FOR CONTROL OF *PECTOBACTERIUMCAROTOVORUM* AT 15% CONCENTRATION USING DIFFERENT ESSENTIAL OILS

The findings demonstrate the creation of an inhibitory zone for peppermint oil's pathogen control. *Pectobacteriumcarotovorum* at a concentration of 15% had the highest level of control (18.33). While in the case of essential oils, control is nonexistent. Different days of therapy with essential oils such as cinnamon, Neem, peppermint and lemon all produced noticeable results. Lemon oil at a 15% concentration after receiving control treatment had the smallest inhibitory zone with a value of (3.73) over the pathogen.

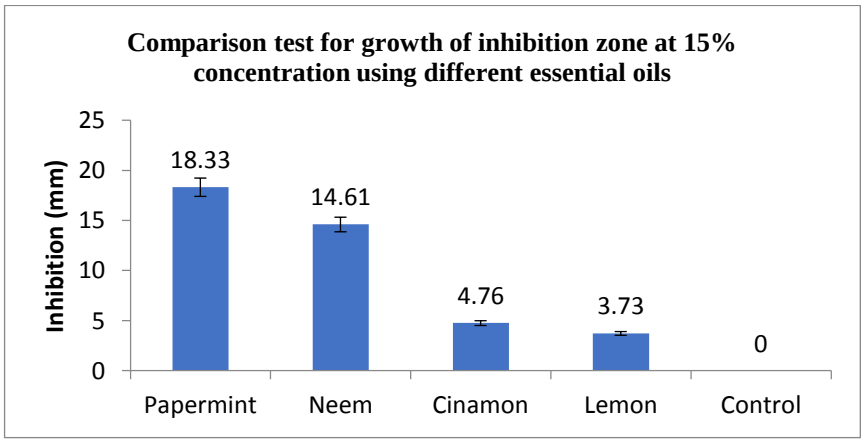

Fig. 4. Comparison test for growth of inhibition zone at 10% concentration using different essential oils

According to the results the pathogen *Pectobacteriumc arotovorum* was most effectively controlled by peppermint oil at a 15% concentration (18.33). The essential oils at a 15% concentration in the control group, however, produced non-significant results. Lemon oil demonstrated a minimal inhibitory zone with a value of (3.73) over the pathogen as compared to the control treatment. Our finding is similar with El-habbak & Refaat (17).

CONCLUSION

In the context of inhibiting soft rot disease, *Menthapiperita, Azadirachtaindica, Cinnamomumverum*, and *Citrus limon* all exhibited inhibition zones. Among these botanicals, *Menthapiperita* demonstrated the highest efficacy, with a remarkable inhibition zone of 18.33% at a concentration of 15%. Conversely, *Citrus limon*

displayed a lower level of effectiveness, with a minimum inhibition zone of 1.79% observed at a 5% concentration.

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Declaration of competing interest:

The authors declared no conflicts of interest.

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