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RESEARCH ARTICLE

Screening for antibacterial activity in selected medicinal plant extracts against *Xanthomonas euvesicatoria*

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ABSTRACT

Background and objectives: The environment and human health have fared badly from the use of synthetic pesticides as a form of pest control. Plants are considered as an alternate pesticide for pest control without sacrificing the environment and their potency on pests.

Methods: Aqueous extracts of (Lavender flower, olive leaves, thuja leaves and fruit, fig milk, clove flower buds, Arabic gum, Borage, Tarragon, Cress seed and Carob tree) were tested against newly isolated *Xanthomonas euvesicatoria* (Acc. No. OP115671) from symptomatic tomato plants.

Results: The in vitro results showed that clove extract is highly effective in inhibiting bacterium growth followed by borage, carob and tarragon respectively. The minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC of clove extract reached 3.125 and 6.25 mg/ml respectively.

Conclusions: This study indicated that aqueous extract of clove can potentially be used as a promising bactericide in the near future.

Key Words: Clove, borage, carob, tarragon, MIC and MBC.

INTRODUCTION

Xanthomonas euvesicatoria is the causal agent of bacterial spot on tomato and pepper world wide spread and cause serious economic losses reach to more than fifteen percent (Jones et al., 1991; Jones et al., 2004). The disease affects all aboveground plant parts specially on leaves and fruits which be most notice (Sun et al 2002). Integrated practices are recommended such as use pathogen-free seeds and seedlings, crop rotation, use of resistance cultivars and chemical control (Lopes and Quezado-Soares, 1997). The use of chemical control as copper fungicides, copper mixture, and antibiotics streptomycin and oxytetracycline may led bacteria to make resistance and it is highly dangerous (Quezado-Duval et al., 2003).

Plant extracts are considered to be the most important sources for pathogen control. Plant extracts are found to be more advantageous over chemical uses due to their health and environmental pollution (Borase et al., 2014). Biocides extracted from plants are environmentally friendly and have no residual effects on consumers, other than the most toxic chemical pesticides (Varma and Dubey, 1999). Plants active constituent content may differ according to plant genetics, as well as to quality of the land where plants grow, the harvesting time, and the extraction methods. Screening traditional plants in the search for novel antimicrobial activity is becoming more popular and playing a very important role nowadays (Dorman and Deans, 2000; Sirajudeen and Muneer, 2014). Due to their antimicrobial and bioregulatory properties, different crude extracts of various species, herbs and other plant materials are becoming increasingly important in the food industry (Tripathi et al., 2004). Different plants and parts of plants were selected such as; Lavender flower (*Lavandula angustifolia*), olive leaves (*Olea europaea*), thuja leaves and fruit (*Thuja occidentalis*), fig milk (*Ficus carica*), clove flower buds (*Syzygium aromaticum*), Arabic gum (*Acacia Senegal*), Borage (*Borago officinalis*), Tarragon (*Artemisia dracunculus*), Cress seed (*Lepidium sativum*) and Carob tree (*Ceratonia siliqa*) based on the previous research on their antimicrobial activity. There have been several reports on the control of human pathogens using these plant extracts, but there is as yet very little reports for the biological

control against plant pathogens; hence, the great interest in studying these indigenous plants.

MATERIALS AND METHODS

Sample collection and pathogen isolation

Tomato fruits and leaves showings typical symptoms of bacterial spot infections by *X. euvesicatoria* were collected from different green houses in Erbil. They were washed and surface sterilized with ethanol 70 percent for 1 minute then washed with sterilized distilled water three-time after drying with filter paper were put on nutrient agar plates. The plates were incubated at 28°C and examined after 24-48 h after that the bacterium colony which has same morphological characteristics sub-cultured and purified on NA and specific media PSA (Peptone Sucrose Agar) incubated at the same condition for further tests.

Pathogenicity Test

Six isolates were selected and tested for their pathogenicity test. The experiment was carried out in a completely randomized design (CRD) one months old tomato seedlings were used with five replications. Bacterial suspension (1×10^6 CFU/ml) was prepared in sterilized distilled water. Tomato seedlings were stem-injected using a sterile hypodermic syringe at the first true leaves. Control was injected with sterilized distilled water and then covered with plastic bag for 24h, after two weeks symptoms were evaluated (Rashid et al., 2016a). The most virulence isolate selected for further experiments.

Pathogen identification

The most pathogenic isolate was cultivated in a nutrient broth at 30°C for 24 hours. A commercial genomic DNA purification kit (Wizard DNA Genomic Purification System 44 Promega, Madison, WI, USA) complete genomic DNA was used for DNA extraction. The 16S RNA gene was amplified using the 8F forward primer (-5-GTGACACGTACACGT-3-) and 1492R reverse primer (-5-ATCGCACGTACACGT-3-) (Turner et al., 1999).

Plant extraction

Eleven different plants were selected and obtained from Research center/Erbil Polytechnic University/Erbil. Ten-gram powder of Lavender flower, olive leaves, thuja leaves and fruit, fig milk, clove flower buds, Arabic gum, Borage, Tarragon, Cress seed and Carob tree were macerated with 100 ml sterilized distilled water in different sterilized flasks for 48 h. The macerate was first filtered through double-layered muslin cloth filtration then filter again with a Whatman filter paper No.1 (ALBERTR) finally they were put in oven 38-40 ˚C until dried.

The percentage of crude extract yield was calculated following Mushore and Matuvhunye (2013) method:

Percentage of yield weight = (W2) – (W1) / (W0) × 100

W0: weight of initial dried sample; W1: weight of empty container; W2: weight of the extract and container

The crude aqueous extracts were readied by dissolving 0.1 g of crude extract in 1 mL of sterile distilled water (100 µg/ ml). Sterile distilled water served as the control.

Antibacterial activity

Antibacterial activity of selected plant extract was determined by well diffusion method on nutrient agar medium (Valgas et al., 2007). One loop of the (24h) *X. euvesicatoria* was taken from the NA plates and transferred to 30 ml test tubes containing the sterilized distil water and vortexes well. The concentration was measured using spectrophotometer (optical density (OD) of 0.1 at 660 nm wavelength) (Rashid, 2021). 150 µl of bacterium suspension (10^6 cfu /ml) was spread out on NA plates using L glass shape. Wells with 0.5 mm diameter were made by the use of a sterile cork borer. Each well was filled with 30 µl of extracts. The plates were incubated at 30˚C for 24 h with four replicates. The inhibition zones were recorded around the wells using a ruler in millimeter (mm).

MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for the most effective plant extract (clove) by Micro-broth dilution assays. 300 µL of Nutrient broth (NB) was filled in each well of polystyrene sterile flat-bottom 96-well plates. The clove extract was filled in first well with 300 µL (100µg/ml) concentration, mixed well then, a 2-fold dilution was carried out from the well 1 to 12. Finally, the bacterial stock solution (106 CFU/ml) was loaded in every well (100 µL) the plates were incubated for 24 h at 30 ◦C.

The MIC was determined when no visible bacterial growth appear (no turbidity is seen). The MBC was determined by subculture each well from MIC essay onto the NA plate and incubated overnight at 28˚C. The concentration which exhibited no bacterial growth was deliberated as MBC (Rashid et al., 2016b).

RESULTS

Isolation and Pathogenicity Test

From the collected samples and different parts of the tomato plants, 6 bacterial isolates were isolated and purified. These 6 isolates were chosen based on morphological and colour, they growth on specific media PSA. The pathogenicity studies showed that from 6 bacterial isolates all the isolates were pathogenic to tomato plant. All the bacterial isolates were re-

isolated from the infected plants but not from the control plants to confirm Koch's postulate. The most virulence isolate was selected for the rest experiments.

Bacteria identification:

The identification was confirmed with PCR amplification with primers 16S of the most pathogenic isolate produced 1400-1500 bp amplicon. A BLAST search in the NCBI database revealed that the 16S sequences of that isolate was 100% identical to *Xanthomonas euvesicatoria* strain KABOb7 GenBank (Acc. No. MT598217). The sequence of the Strain Mt7 was deposited in GenBank (Acc. No. OP115671).

Crude extract yield

After dryness tarragon leaves gives the highest percentage of yield 19.6 followed by clove and cress seeds 16.8 and 14.4 respectively. Thuja fruit gave the lowest percentage of yield 6.9 (fig 1).

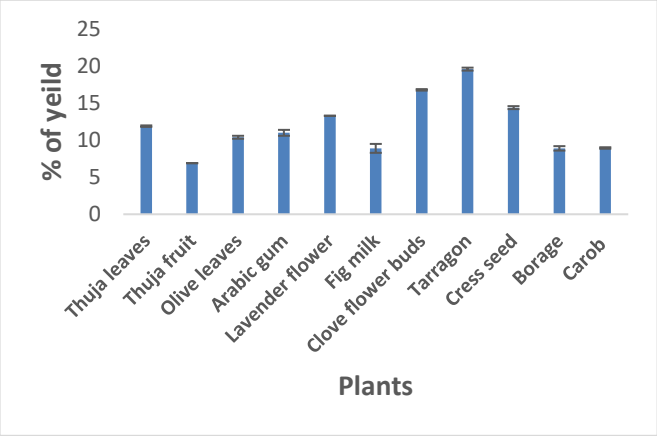

Fig (1): Percentage of plant crude extract yields

Antibacterial Activity

From eleven different plant extracts clove extract showed highest antibacterial activity against *Xanthomonas euvesicatoria* with 23 mm (fig 2). Also, borage flower gave 14 mm inhibition zone followed by tarragon and Carob were 12mm. The rest of the plant extracts did not give any antibacterial activity against tested bacterium (table 1).

Table 1: Antibacterial activity of different plant extracts against *X. euvesicatoria* using well diffusion method

	Plants	Inhibition zones (mm)
1	Thuja leaves	0
2	Thuja fruit	0
3	Olive leaves	0
4	Arabic gum	0
5	Lavender flower	0
6	Fig milk	0
7	Clove flower buds	23
8	Tarragon	12
9	Cress seed	0
10	Borage	14
11	Carob	12

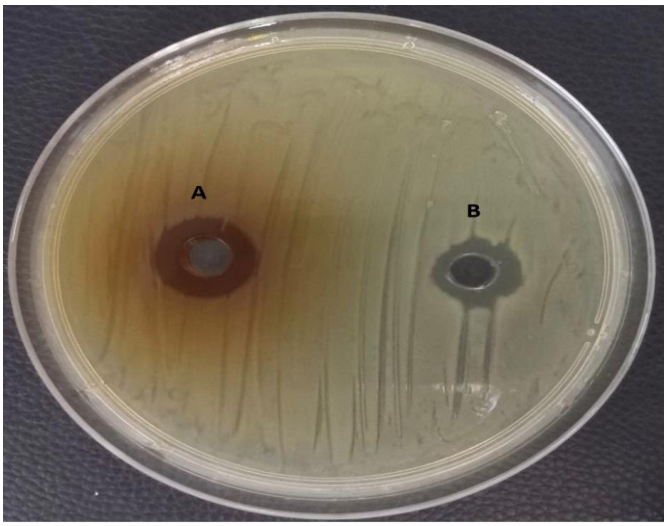

Fig 2. inhibitory effects of the clove aqueous extract against *X. euvesicatoria* (A: clove extract; B: Borage)

MIC and MBC

The MIC and MBC of the clove crude extracts against the tested bacterium using the broth micro-dilution method were measured. The MIC was 3.125 mg/ml and MBC was 6.25 mg/ml.

DISCUSSION:

The antibacterial activities of different plant species have been studied. The crude extracts of clove and Starflower exhibited antibacterial properties against *X. euvesicatoria*. Different researchers reported the antimicrobial activity of Borage against human and foodborne pathogens. Karimi et al. (2018) reported the antimicrobial activity of borage methanolic, ethanolic and aqueous extracts against common human and foodborne pathogenic bacteria. Abolhassani (2004) reported the aqueous extract of borage flowers in vitro showed antibacterial activity against Staphylococcus aureus 8327. Also, Al-Rimawi et al. (2021) reported antibacterial activities of borage seeds oil and leaves extracts against *Staphyloococcus aureus*.

Syzygium aromaticum commonly known as clove was used in traditional medicine against human -pathogenic (Goyal and Ayeleso, 2018). Clove oil extract reported that inhibit bacteria and yeast such as *Pseudomonas aeruginosa, Salmonella species*,

Staphylococcus aureus, Escherichia coli, Corynebacterium species, Bacteroides fragilis, Streptococcus pyogenes and *Candida albicans* (Nzeako et al., 2006). Ethanol, aqueous extracts, and essential oils of cloves were showed antibacterial activity against twenty-one food borne pathogens: *Staphylococcus aureus* (4 strains), *Escherichia coli* O157: H7 (6 strains), *Listeria monocytogenes* (5 strains), *Salmonella Enteritidis* (4 strains), *Bacillus cereus* and *Vibrio parahaemolyticus* and 5 food spoilage bacteria: *Alcaligenes faecalis, Pseudomonas putida, P. aeroginosa*, and *Aeromonas hydrophila* (2 strains) (Hoque et al., 2008). In addition, methanolic clove extract tested against food borne pathogens gram positive culture *Staphylococcus aureus* and gram-negative culture *E. coli* and *Pseudomonas aeruginosa* (Pandey and Singh, 2011). Borase et al. (2014) evaluated the antimicrobial potential of ethanolic and water extracts clove, on (*Staphylococcus aureus Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis and Vibrio parahaemolyticus* and *Candida albicans*). The extracts displayed both antibacterial and antifungal activities against all tested microorganisms. Different studies reported the antibacterial activities of clove extracts against human pathogens. Mirpour et al. (2015) used methanolic clove extract to control Streptococcus mutans and *S. salivarius* which cause oral infection. Aqueous and ethanolic extracts of clove had inhibitory activity against *S. aureus, P. aeruginosa* and *E. coli*. Concentration between 500-700µg/ml showed good inhibitory effect against all tested bacteria (Mostaqim et al., 2019).

Also, there are some reports on using clove extract against plant pathogens. Oil clove was determined antibacterial activity against group of plant pathogen both positive and negative bacteria, the most sensitive was *Ralstonia solanacearum* that cause bacterial wilt of tomato and geranium (Huang and Lakshman, 2010). Clove extract show better testing result to suppress damping-off of vegetable seedling (tomato, eggplant, chilli) and increased the seed gemination of these plants (Islam and Faruq, 2012). Clove extract was tested on tomato plants whose inhibiting effect was assessed on Oidium sp. spores additionally induce plant defense for their ability expression of different defense gene (Malo et al., 2017). Thabet and khalifa (2018) investigated clove oil in different concentration against *Fusarium oxysporum, F. semitectum F. solani*, and *Rhizoctonia solani*, noticed decrease the mycelia growth and change on fungi morphological features especially at 4% concentration resulted a highly significant decrease. Šernaitė et al. (2020) Showed at their study the ability of clove extract to inhabit Botrytis cinerea which cause grey mold on strawberry. Essential oils of clove tested in vivo and in vitro against *X. euvesicatoria*, which results to loss integrity of bacterial cell wall leading to cell death after changing of morphological and physiological (Lucas et al., 2012a). In recent study clove extract showed effective antimicrobial results to kill ten of potato phytopathogens that cause great economical losses. The lowest MBC/MFC values were recorded *Rizoctonia solani, Phoma exigua* and *Alternaria solani* (6.3 mg/mL), *Streptomyces scabiei, Fusarium oxysporium, Fusarium sambucinum, Alternaria tenuissima* and *Colletotrichum coccodes* (12.5 mg/mL) while *Pectobacterium carotovorum* was the highest value (25 mg/mL) (Steglińska et al., 2022).

CONCLUSION:

Aqueous extracts of different plants were tested against *X. euvesicatoria*, clove flower buds, borage, carob tree and tarragon showed antibacterial activity. As compared to other tested plants, clove extract was found to exhibit the most potent antibacterial activity. This extract could be used as a source for a natural product to bring tomato bacterial spot disease under control.

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