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BIO-CONTROL EFFECT OF *ERUCA SATIVA* MILL. OIL AGAINST THE HAZARDOUS FOOD BORNE PATHOGENS

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A B S T R A C T

Antimicrobial activity of *Eruca sativa* Mill. oil was evaluated against phytopathogenic bacterial species (*Xenorhabdus luminescens, Acinetobacter sp., Bordetella pertussis, Ensifer adhaerens, Pseudomonas syringae, Acidovorax temperans, Xanthomonas axonopodis*) and fungal species (*Alternaria alternata, Dreschlera halodes, Aspergillus nidulans, Acremoniun kiliense, Fusarium oxysporum, Curvularia clavata, Rhizopus oryzae*). Antifungal activity was determined on MEA while antibacterial activity on NA media plates to measure the effects of oil. The antimicrobial activity was tested by well diffusion method *in vitro*. *E. sativa* oil was found to be highly active against all fungal isolates tested as compared to bacterial isolates. Results showed evidence of high antibacterial activity against *X. luminescens* with inhibition zone of 3.1 cm. *E. adhaerens* and *A. temperans* exhibited least resistance against oil with 1.4 cm and 1.7 cm zone of inhibition respectively. The oil showed high antifungal activity in the range of 6.0-6.8cm inhibition zone against *D. halodes*, *C. clavata*, *R. oryzae* and *A. nidulans* whereas least active against *F. oxysporum* with 1.1cmzone of inhibition. The antimicrobial components from this oil can be used as an alternative to develop novel pesticides by replacing some chemical commercial antifungal and antibacterial for the plant diseases.

Keywords: *E. sativa* oil, antimicrobial activity, phytopathogens.

INTRODUCTION

Plants and herbal extracts have formed important position in modern medicine, due to their chemical and medicinal contents found in natural from. History of herbal remedies is very old; there are many medicinal herbs and spices, which find place in day-to-day uses. Authentic effects of any plant extracts on a particular plant disease prompt us to screen indigenous plants those also having potential for antioxidant and antimicrobial activity. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem. This compelled the scientists to search out new drugs from plant origin. Plant derived antimicrobial compounds might inhibit pathogens' growth through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes. There is a need to evaluate the herbs scientifically for their antimicrobial activity against the antibiotic-resistant microorganism in order to develop new drug from plant origin (Khoobchandani *et al.,* 2010). Essential oil is more or less volatile material isolated from an odoriferous plant of a single botanical species and it carries a distinctive scent, or essence, of the plant. Essential oil differs from the fatty or fixed oils both in composition and properties. Essential oils have no fixed structure as they are the mixture of different components. Medical applications of these oils range from skin treatments to remedies for cancer (Mahmood *et al.,* 2008).

Eruca sativa Mill. locally known as *Taramira* belongs to the family Brassicaceae is grown in different parts of Indo-Pak subcontinent and Middle East. It is a low growing, annual oilseed crop with dull green, deeply cut compound leaves. It is minor oil crop and used in traditional medicines as remedies for different diseases. There is sporadic information available about phytochemistry and bioactivity of this oily crop. It is known as diuretic, anti-inflammatory and affects on blood circulation by various authors (Rani *et al.,* 2010; Sartoratto *et al.,* 2004). Its oil is used for pickling, after

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aging to reduce the acridity, as a salad or cooking oil. The oil is also used as massage oil and to soothe the skin. The seed cake, a byproduct of oil production, is also of use as animal feed (Gulfraz, *et al.,* 2011). The present study was conducted to inhibit the growth of different fungal and bacterial phytopathogens by using *E. sativa* oil. This is the first attempt to study the antifungal and antibacterial activity of *E. sativa* oil.

MATERIAL AND METHODS

Extraction of essential oil: Sixty gram of fresh leaves of *E. sativa* was subjected to steam distillation in a Dean and Stark assembly for three hours. Two layers were formed, upper organic layer of oil and lower aqueous layer of water. Lower aqueous layer was discarded and upper layer of oil was collected. Then oil was further purified by solvent extraction using diethyl ether (25 ml). Bright yellow oil was further refined with Na_2SO_4 in order to remove traces of water if present. Then oil sample was stored in dark brown bottle and was kept in refrigerator. (Mahmood *et al.,* 2008).

Procurement of phytopathogenic strains: A total of fourteen phytopathogens of bacterial and fungal strains used for this study were obtained from First Fungal Culture Bank of Pakistan (FCBP) (Table 1). Cultures were revived on malt extract agar (MEA) and nutrient agar (NA) media for fungal and bacterial species, respectively.

Table 1. List of phytopathogens of bacterial and fungal strains.

FCBP #	Fungal Strains	Source	FCBP #	Bacterial Strains	Source
1174	*Alternaria alternata*	Tomato fruit	119	*Xenorhabdus luminescens*	*Citrus sinensis* fruit
1133	*Dreschlera halodes*	Tomato fruit	334	*Acinetobacter* sp.	*Citrus sinensis* fruit
1121	*Aspergillus nidulans*	*Citrus sinensis* fruit	333	*Bordetella pertussis*	*Citrus sinensis* fruit
1045	*Acremoniun kiliense*	Citrus fruit	335	*Ensifer adhaerens*	*Citrus sinensis* fruit
1020	*Fusarium oxysporum*	Lemon fruit	010	*Pseudomonas syringae*	*Pyrus malus* fruit
1026	*Curvularia clavata*	Falsa fruit	227	*Acidovorax temperans*	*Citrus sinensis* fruit
0985	*Rhizopus oryzae*	Citrus fruit	001	*Xanthomonas axonopodis*	*Citrus sinensis* fruit

Standardization of inoculum The test organisms were sub-cultured onto fresh plates of MEA for 5 days at 25 ºC and NA for 24 hrs at 37 ^{0}C for fungi and bacteria, respectively. Colonies from these plates were suspended in 5 ml of Barium chloride (1 %) to a turbidity matching 0.5 McFarland standard (10^8 colony forming units (CFU)/ml).

Preliminary screening for antimicrobial activity

Antifungal assay: The *in vitro* tests were carried out to measure the antifungal efficacy of *E. sativa* oil on radial growth of fungal isolates by agar well diffusion method (Mushtaq *et al.,* 2012). Eighty microlitres (80 µL) of the standardized inoculum (10^8 CFU/mL) of each test fungus was spread with the help of sterile spreader on to a sterile MEA plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of 0.8 cm diameter was used to bore wells in the agar plates. Subsequently, a 60µL volume of oil was introduced in wells into MEA plate. The plates were allowed to stand for at least 1 hr for diffusion to take place and then incubated at 25 ºC for 5 days. For control, $DMSO_4$ was filled in the wells against each tested fungal isolate. The zone of inhibition was recorded to the nearest size in cm. The experiment was carried out in three replicates.

Antibacterial assay: The same method as for fungi was adopted for antibacterial assay of *E. sativa* oil against all the tested bacterial species. Instead of malt extract agar, nutrient agar was used. The bacterial suspension (McFarland 0.5) was spread on the agar surface using sterile cotton swab. Then a well of 0.8cm was made in the medium using sterile cork borer, 60 µl of oil was poured into these wells and plates were incubated at 37 ºC for 24 hrs. In control, $DMSO_4$ was filled in the wells against tested phytopathogenic bacteria. The zone of inhibition was recorded to the nearest size in cm. The experiment was carried out in three replicates.

Antifungal and antibacterial activity Index was calculated as:

Antimicrobial Index (AI) = Da/Db – 1

Where: Da is the diameter (cm) of the growth zone in the experimental dish and Db is the diameter of the growth zone in the control dish.

Determination of minimum inhibitory volume: The maximum inhibition diameter of the target fungal and bacterial species was checked again by minimum inhibitory volume (Ali *et al.,* 2012). The fungal and bacterial suspensions in different volumes of 10, 20, 30, 40, 50, 60, 70, 80, µL and 100 µL concentrations were

loaded into sterile well on MEA and NA medium separately. Then plates were allowed to incubate for 5 days at 25 ºC for fungi and for 24 hrs at 37 ^{0}C for bacteria, the minimum inhibitory volume effect was determined by measurement of the inhibition zone diameters.

Statistical evaluation: The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates.

RESULT AND DISCUSSION

The present study with *E. sativa* oil gave varied results against fungal and bacterial pytopathogens *in vitro* (Tables 2).

Antibacterial assay: In present study *E. sativa* oil showed evidence of high antibacterial activity against *X. luminescens* with 35 % inhibition. *E. adhaerens and A. temperans* had least resistance against oil with 15.0 and 19.0 % inhibition respectively. Whereas, *Acinetobacter* sp. (27.0 %), *B. pertussis* (27.0 %), *X. axonopodis* (24 %) and *P. syringae* (20.0 %) moderately controlled by *E. sativa* oil.

Antifungal assay: *E. sativa* oil was found to be highly active against all fungal isolates tested as compared to bacterial isolates. The oil showed high activity in the range of 60.0-67.0% inhibition against *D. halodes*, *C. clavata*, *R. oryzae* and *A. nidulans.* On the other hand, *A. kiliense* (49.0 %) and *A. alternata* (38.0 %) were moderately controlled by oil. Whereas *E. sativa* oil was found to be least active against *F. oxysporum* with 13.0 % inhibition.

Table 2. Antimicrobial activity of E. sativa oil against fungal and bacterial phytopathogens

Antimicrobial Activity of *E. sativa* oil (Taramira) in cm					
	Microbial Growth in Control	Inhibition zone	Experimental growth zone	Index	%age of Inhibition
Bacterial Pathogens					
Xenorhabdus luminescens	9.0±0.0	3.1±0.05	5.9±0.03	0.35	35.0
Acinetobacter sp.	9.0±0.0	2.4±0.01	6.6±0.03	0.27	27.0
Bordetella pertussis	9.0±0.0	2.4±0.06	6.6±0.03	0.27	27.0
Ensifer adhaerens	9.0±0.0	1.4±0.05	7.6±0.03	0.16	15.0
Pseudomonas syringae	9.0±0.0	1.8±0.18	7.2±0.18	0.20	20.0
Acidovorax temperans	9.0±0.0	1.7±0.08	7.3±0.08	0.20	19.0
Xanthomonas axonopodis	9.0±0.0	2.1±0.20	6.9±0.20	0.24	24.0
Fungal Pathogens					
Alternaria alternata	9.0±0.0	3.5±0.02	4.4±0.13	0.93	38.0
Dreschlera halodes	9.0±0.0	6.8±0.04	7.1±0.02	0.78	67.0
Aspergillus nidulans	9.0±0.0	6.0±0.12	6.6±0.07	0.71	60.0
Acremoniun kiliense	9.0±0.0	4.0±0.45	5.5±0.05	0.60	49.0
Fusarium oxysporum	9.0±0.0	1.1±0.08	5.2±0.14	0.50	13.0
Curvularia clavata	9.0±0.0	6.5±0.13	6.8±0.05	0.75	64.0
Rhizopus oryzae	9.0±0.0	6.1±0.09	6.5 ±0.05	0.72	61.0

Comparison of phytopathogens against *E. sativa* oil with Minimum Inhibitory volume: The volume of *E. sativa* oil was employed in the range of 10 to 100 µL for *X. luminescens* and *D. halodes* (Table 2). Table 3 also shows that *D. halodes* detected the highest inhibition by *E. sativa* oil as compared to *X. luminescens*. The minimum inhibitory volume of *E. sativa* oil that completely stopped the growth of *X. luminescens* was <40 µL and above. On the other hand, only volumes of <20 µL of *E. sativa* oil completely inhibit the growth of phytopathogen in case of *D. halodes*.

Essential oils possess antimicrobial properties and are supposed due to the presence of important components mostly phenolic in nature which exert membrane-damaging effects to microbial strains and stimulates leakage of cellular potassium ions which is responsible for a lethal action related to cytoplasmic membrane damage (Tadtong *et al.,* 2009).Herbal medications in the form of oils have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to natural preparations as compared to synthetic pharmaceuticals. With the reduced costs of essential oils preparation, makes the search for natural therapeutics an attractive option. Therefore these oils possess antibacterial and

antifungal activity against various pathogens of plants (Sartoratto *et al.,* 2004; Sabulal *et al.,* 2006; Srinivasan *et al.,* 2009; Rani *et al.,* 2010; Gulfraz *et al.,* 2011). In this connection, the present study was conducted to evaluate the antimicrobial activity of *E. sativa* oil against

Table 3. Minimum inhibitory volume of E. sativa oil against fungal and bacterial phytopathogens.

Minimum Inhibitory volume	Diameter of Inhibition Zone (cm)	
	X. luminescens	*D. halodes*
10 µL	NA	NA
20 µL	NA	NA
30 µL	NA	0.43±0.03
40 µL	NA	1.20±0.16
50 µL	1.90±0.03	2.10±0.03
60 µL	2.10±0.13	3.00±0.03
70 µL	2.90±0.06	3.60±0.10
80 µL	3.70±0.03	4.00±0.03
90 µL	3.90±0.03	4.10±0.03
100 µL	4.00±0.03	4.40±0.03

The contents of *E. sativa* oil depend on many factors including maturity of the seed and the degree of plant irrigation. Whereas, it contains important secondary metabolite such as flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid and those are used as remedies of many diseases and frequently required in traditional medicines. Erucic acids are present in high concentration in *E. sativa* oil, those are responsible for antibacterial activity that could be used for the preparation of drugs required for plant, human and animal health (Gulfraz *et al.,* 2011). In the present investigation, the antimicrobial activity against various test fungal and bacterial plant pathogens was studied in comparison with $DMSO_4$ by well diffusion method *in vitro*. In addition, Rani *et al.,* (2010) also exhibited the antimicrobial activity of *E. sativa* against two Gram negative bacteria and four fungal species and results displayed highest antibacterial activity while fungal species showed variable degrees of inhibition even at lower concentration. Furthermore, Gulfraz *et al.,* (2011) also studied the efficacy of *E. sativa* oil against Gram negative and positive bacteria thus results showed strong antibacterial activity which was similar to our report. According to its antimicrobial activity, this oil might be used as an active ingredient in antimicrobial formulations. Hence, it may be the protective substance in *E. sativa* oil. So the elucidation of active constituents in oil may provide useful lead to the development of new phtopathogens. As the work for the development of herbal medicines is in progress worldwide, the present report will help in isolation of new products. Besides, the same may also be used for the treatment of plant pathogenic fungi and bacteria as conventional method. and effective antimicrobial agents. In order to find an alternative approach for discovery of medicinal components, further studies are needed for more extensive evaluations of the biological properties of *E. sativa* oil.

CONCLUSION

The results from the present study offer a scientific proof for the traditional use of the *E. sativa* oil. In antimicrobial assay, the antibacterial/antifungal activity of oil is investigated by well diffusion method *in vitro*. The oil showed a strong inhibitory effect against the growth of bacterial and fungal isolates which are associated with different plant diseases. The antimicrobial activity shown by the oil may be due to a synergistic effect of various components present in it. It is usually found that such combined effect of various components is responsible for the therapeutic activities of *E. sativa* oil. However, this needs further investigation and the work is in progress.

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