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COMPARISON OF ANTIMICROBIAL ACTIVITIES, HERB AND ESSENTIAL OIL PRODUCTION OF *Thymus vulgaris* L. AND *T. serpyllum* L. IN BALOCHISTAN, PAKISTAN

Shahina Momin[1], Shazia Irfan[1], Sarfraz Ahmad[2], Misbah Manzoor[1] and Hina Irfan[3]

ABSTRACT

Studies were carried out at Balochistan Agricultural Research and Development Centre formally Arid Zone Research Centre (AZRC), Quetta to compare the production, essential oil and antimicrobial activities of two Thyme species, Thymus vulgaris L. and T. serpyllum L. Syn. T. linearis Bentham. Production of T. vulgaris was significantly higher than the T. serphyllum. Fresh and dry production (leaves & twigs) of T. vulgaris was recorded 2932 kg and 2082 kg ha^{-1} while the production of T. serpyllum was 168 kg ha^{-1} and 113 kg ha^{-1}. Essential oil content of T. serphyllum was higher both in fresh and dry plant samples. Essential oil content in T. vulgaris was 0.33% and 0.46% in fresh and dry samples while the essential oil content in T. serphyllum was 0.58% and 0.87% in fresh and dry plant samples, respectively. Antibacterial activity was tested using the Kirby Bauer Bioassay method against the common pathogens Escherichia coli cultured on MHA media. Three different concentrations of leaf extracts (0.5g, 1g, 1.5g) were used for three different time periods (24 hrs, 48 hrs, 72 hrs). One gram leaf extract of T. vulgaris for 48 hours and 0.5 g extract of T. serpyllum for 24 hours were found most effective in producing maximum inhibition. Both species have multi-purpose uses like medicinal properties, culinary herbs, essential oil source and herbal tea and have potential for large scale cultivation in highlands of Balochistan.

Key words: Thyme, production, essential oil, Bioassay, *E. coli*.

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[1]Dept. of Botany, [3]Dept. of Environmental Sciences, Sardar Bahadur Khan Women's University, Quetta, Pakistan
[2]Pakistan Agricultural Research Council, Islamabad, Pakistan.
*Corresponding author's email: shaziairfan66@yahoo.com

INTRODUCTION

Thyme is an ever green perennial herb of the Labiatae family native to Mediterranean area and many wild species are reported in Asia and Central Europe (Anwar and Masood, 1998; Khan and Khatoon, 2008; Shinwari, 2005). Ecological conditions play a major role in the cultivation of medicinal plants and active ingredients (Yusuf *et al.*, 2005). Thyme is known as a medicinal plant from the early civilizations of the Mediterranean Basin. It has a very long history of folk use for a wide range of ailments. It is an excellent tonic and is used in treating respiratory diseases, colds, flu, cough, sore throat, bronchitis, asthma, indigestion, gastritis, depression and lethargy. (Low *et al*., 1994). Thyme medicinal properties are anti-rheumatic, antiseptic, antispasmodic, bactericidal, carminative, diuretic, and expectorant (Dorman and Deans, 2000; Jamroz *et al.*, 2003; Sarica *et al.*, 2005; Tucker, 2002). Leung (1980) reported that Thyme and its oil has been used as fumigant antiseptic antioxidant, and mouth washes. Thyme is often used to flavor meats, soups and pickles (Daferera *et al.*, 2000). In many countries Thyme is cultivated both for seasoning and for its volatile oil. Thyme production is geographically widespread (Akgul, 1993; Moldão-Martins *et al.*, 1999). Thyme is also an important nectar source plant for honey bees (Di-Pasqua *et al.*, 2005; Suppakul *et al.*, 2003; Zargary, 2006).

Thyme is rich in essential oils and its main constituents are Thymol and Carvacrol. These are the active ingredients responsible for most of the medicinal properties (Baranauskiene *et al.*, 2003; Masango, 2005; Zizovic *et al.*, 2005). Thyme essence especially the phenolic components thymol and carvacol showed antibacterial activity against Gram-positive and Gram-negative bacteria (Dobre *et al.,* 2011; Mith *et al.,* 2014; Nevas *et al.*, 2004). However, gram positive bacteria *Clostridium botulinum* and C. *perfringens* appeared to be more sensitive than the gram-negative organisms. Experiments were conducted during 2008-2009 to determine the production, essential oil and antimicrobial activity of two Thyme species (*Thymus vulgrais* and *T. serpyllum*).

MATERIALS AND METHODS

Plant material

Samples of thyme were collected during October to November, 2009 from Balochistan Agricultural Research and Development Centre formally Arid Zone Research Centre (AZRC) experimental filed (*Thymus vulgaris* L.) and Ziarat (*Thymus serphylum* L.) by Quadrate method. Identification of the species was confirmed and a voucher specimen was preserved in Sardar Bahdur Khan Women's University Herbarium. In each $1m^2$ Qudrate, number of plants, plant height (cm),

plant fresh weight (g) and one week shade dry plant weight (g) were recorded. The fresh and shade dried aerial parts (1000g) were submitted to Hydro distillation for 2 h using Stem Distillatory apparatus. The essential oil was collected, dried over anhydrous sodium sulphate and essential oil % age was calculated.

Antimicrobial Activity

Kirby- Bauer method was used for screening the antimicrobial potential of the plant extract of *Thymus vulgaris* and *Thymus serpyllum* leaf extract on *Escherichia coli.* Plant extracts were prepared by soaking 0.5 g, 1g and 1.5g leaf powder in100 ml distilled water and kept them for 24 hours, 48 hours, and 72 hours, respectively. Mueller Hinton agar was used for culturing *E. coli* under aseptic conditions. Five filter paper discs (5 mm in diameter) saturated with extract was placed on surface of each inoculated plate. The plates were incubated at 37 °C for 24 h. After this period, it was possible to observe inhibition zone. After 48 hours the inhibition zone were measured in millimeter.

RESULTS AND DISCUSSION

Production and Essential Oil

Table-1 showed herb production and essential oil %age of *Thymus vulgaris* and T. *serphyllum*. Fresh and dry production of *T. vulgaris* was recorded 2932 and 2083 kg ha^{-1} while the fresh and dry production of *T. serphyllum* was 168 and 113 kg ha^{-1}, respectively. The production of *T. vulgaris* was significantly higher than *T. serpyllum*. However, the essential oil content of *T. serphyllum* was higher than the *T. vulgaris* (Table-1).

Table-1. Fresh, Dry Production and Essential Oil of *Thymus vulgaris* and *T. serphyllum.*

Plant species	Production (kg ha^{-1})		Essential oil %	
	Fresh	Dry	Fresh leaves	Dry leaves
Thymus vulgaris	2932	2082	0.33	0.46
Thymus serphyllum	168	113	0.58	0.87

The production of *T. vulgaris* was higher as compared to *T. serphyllum* due to its cultivation and better management practices. Thyme is a cold and drought tolerant species and has cultivation potential in highlands of Balochistan(Ahmad *et al.*, 2008). Omidbaigi and Nejad (2000) reported that nitrogen fertilizer had a significant effect on the dry-matter production of Thyme yield. The herb yield increased from 671.88 kg to 1021 kg ha^{-1} as a result of 150 kg ha^{-1} nitrogen dose.

Antimicrobial Activity

Mean inhibition and standard error of inhibition zone of the leaf extract of *Thymus* spp. against *E.coli* is presented in Table-2. It was observed that when 0.5 g of aqueous leaf extract of *T. vulgaris* was kept for 72 hours, exhibited highest value of mean inhibition (3.8 ± 1.37) as compared to 48 and 24 hours. One g of aqueous leaf extract of *T. vulgaris* when kept for 48 hours, exhibited the highest value of mean inhibition (7.2± 0.982) as compared to 72 and 24 hours and 1.5 g of aqueous leaf extract of *T. vulgaris* exhibit highest value of mean inhibition in 24 hours (2.133 ± 1.490) as compared to 48 and 72 hours. When 0.5 g of aqueous leaf extract of *T. serpyllum* was kept for 24 hours, it exhibited the highest value of mean inhibition (7.733 ±1.677) as compared to 48 and 72 hours. One g of aqueous leaf extract of *T. vulgaris* when kept for 48 hours, exhibited highest value of mean inhibition (6.533 ±1.111). When 1.5 g of aqueous leaf extract of *Thymus vulgaris* was kept for 24,48 ,72 hours, it exhibited highest value of mean inhibition in 48 hours (6.8 ± 1.079) as compared to 24 and 72 hours.

Table-2. Mean inhibition zone and Standard Error of aqueous leaf extract of *Thymus vulgaris* L. against *E.coli.*

Time	*Thymus vulgaris*			*Thymus serphyllum*		
	Concentrations			Concentration		
	0.5 g	1.0 g	1.5 g	0.5 g	1.0 g	1.5 g
24 hrs	2.066± 0.662	2.133± 0.662	2.133 ± 1.490	7.733 ±1.677	6.466 ± 1.5009	4.933± 0.923
48 hrs	2.266 ± 0.658	7.2± 0.982	1.266 ± 0.485	6.333 ± 0.574	6.533 ± 1.111	6.8 ± 1.079
72 hrs	3.8 ± 1.37	1.8 ± 0.678	1.266 ± 0.485	3.533 ± 0.611	3.933 ± 0.560	4.8 ± 0. 442

Concentration and time limit against the test organism (*E.coli)* proved to play a decisive role in producing maximum growth inhibition of bacteria. Moderate time and concentration of leaf extract is more effective against *E.coli* and if, low concentration is kept for more time limits, also shown good effect. Several researchers used thyme plant against many other strains of bacteria and microbes (Maksimović *et al.*, 2008; Mironescu and Georgescu, 2008; Solomakos *et al.*, 2008). Similar results were found with methanol extracts of five Cameroonian edible plants namely *Colocasia esculenta*, *Triumfetta pentandra*, *Hibiscus esculentus*, *Canarium schweinfurthii* and *Annona muricata* against a panel of 19 multidrug resistant Gram-negative bacterial strains by Dzotam *et al.,* 2016 and aqueous extracts of *T. vulgaris, Lavandula angustifolia, Melissa officinalis, Ocimum basilicum,*

Allium schoenoprasum and *Petroselinum crispum* were tested on five gram negative bacteria – *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Hafnia alvei* and *Raoultella terrigena* by Dostalova *et al.* (2014) and various solvents and water extracts of *Aloe vera*, neem, bryophyllum, lemongrass, tulsi, oregano, rosemary and thyme on 10 multi-drug resistant clinical isolates from both Gram-positive and Gram-negative bacteria and two standard strains including *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 by agar well diffusion method by Dahiya and Purkayastha (2012). Many research studies on thyme are carried out for its antifungal activity. Fan and Chen (2001) reported that the alcohol and ethanol extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *Bacillus subtilis*, *S. sonnei,* and *E. coli.*

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