Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(8); 1148-1152

ISSN: 0975-4873

Research Article

Study of the Efficiency of *Trachyspermum ammi* L. Essential Oil for its Application in Active Food Packaging Technology

Rency Elizabeth*, Akshay Shah

Department of Biotechnology, Mithibai College of Arts, Chauhan Institute of Science and Amrutben Jivanlal College of Commerce & Economics, Vile Parle (West), Mumbai- 400 056, Maharashtra, India

Received: 24th June, 17; Revised 29th July, 17, Accepted: 15th Aug, 7; Available Online:25th Aug, 17

ABSTRACT

The interest in essential oils and their application in food packaging and preservation has been amplified in recent years by an increasingly negative consumer perception of synthetic preservatives Close to one third of the world's food supply is wasted annually. As a major contributor, food spoilage represents an environmental problem as well as an ethical issue. Besides physical damage, browning and staling, molds and yeasts cause significant food waste. Protecting foods from spoilage is essential in order to reduce food waste and ensure safety for consumers. However the current methods employed for preservation carry serious drawbacks which have implications on the health and well being of the consumer. There is a strong need to replace synthetic methods with the use of botanicals. The food industries are developing new packaging systems (active packaging) through the incorporation of essential oils. Essential oils are naturally occurring, degradable, and cheaper than chemical preservatives. Ajwain is a commonly used spice and has been used as a traditional medicine in Indian culture. The current study identifies the fungi toxic potential of essential oil of Ajwain (*T. ammi*) against common food spoilage fungi and its application as a natural preservative and a prospective component of active packaging and micro atmosphere preservation systems. Using bread, a commodity commonly susceptible to fungal contamination in a modelling system aids in understanding the large scale and realistic application of the system developed. Further studies need to be carried out on the synergistic action of essential oil of *T. ammi* with other essential oils and other hurdle techniques. The organoleptic and sensory changes caused due to its strong aroma need to be tested further.

Keywords: Essential oils, active packaging, *T. ammi*, vapour phase.

INTRODUCTION

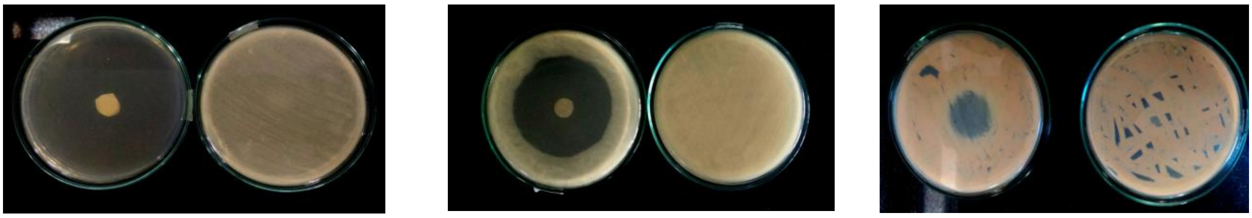
In most of the food stuffs which have a low pH, low water activity or a high concentration of sugars or salts the spoilage bacteria with some exceptions, are usually absent because the characteristics of these food cater for conditions which are unfavourable for their growth. However, food spoilage yeasts and molds can tolerate a wider range of extremes and thus are able to grow under these circumstances and cause deterioration of various products such as fruit and vegetable juices, purees, soft drinks, pickles, dairy products, bread and other baked commodities, dried fruits, sausages, etc. These fungi are predominantly responsible for aflatoxin contamination of crops prior to harvest or during storage[1].

Many strategies are taken intending to prevent fungal growth and further mycotoxin production and food contamination. Heat treatment and antiseptic packaging exclude yeast and mold spoilage as long as the packaging is intact. Products which cannot be pasteurized are usually treated with weak acid preservatives. However the use of these chemical, physical or biological treatments requires sophisticated equipment and expensive chemicals or reagents. Chemical preservatives also present some problems. It was recently reported that benzene can be formed from benzoic acid in foods by decarboxylating action of spoilage microorganisms.

In the wake of such discoveries there is a strong consumer demand to avoid or diminish the use of synthetics in foods. The use of natural plant extracts provides an opportunity to avoid chemical preservation, thus the search for new antifungal material from natural sources for food preservation has increased.

Plants known as condiments are traditionally used to enhance taste or aroma of food and their essential oils (EO) represent a complex mixture of natural substances. Essential oils are known to possess antibacterial and antifungal activity and have been empirically used as antimicrobial agents but the spectrum of activity and mechanisms of action remain unknown for most of them[2]. Essential oils can be added directly to food or can be applied in vapour phase as a part of active packaging system. For essential oils of plants used as condiments the antimicrobial activity seems to be associated with phenolic compounds and the effect is related mainly with changes in the permeability and integrity of cell membrane. The oils and their components increase lag phase and diminish the maximum cell count in stationary phase of mold growth. The colony forming ability of the molds was also reduced or stopped by the essential oils[3,4,5].

**Author for Correspondence: rencyelizabeth@rediffmail.com*



A: Test and control plates after 2 days of incubation)

B: Test and control plates after 4 days of incubation

C: Test and control plates after 6 days of incubation

Figure 1 A, B, C: Antifungal activity of essential oil of Ajwain (*Trachyspermum ammi)* in vapour phase against fungal isolate after 2 days 2, 4 days and 6 days of incubation.

The observations recorded for the zone of inhibition are as follows:

Table 1: Zone of inhibition of Antifungal activity of essential oil of Ajwain (*Trachyspermum ammi)* in vapour phase against fungal isolate after 2 days 2, 4 days and 6 days of incubation.

Incubation Period (in hours)	Zone of Inhibition (in mm)
48 hours	90 mm (complete inhibition)
96 hours	48 ±3mm
144 hours	13± 2 mm

Table 2: Comparison of fungal growth on test and control plates.

Incubation Period (in hours)	Fungal growth on control plate (in mm)	Fungal growth on test plate (in mm)
48 hours	82 ± 4 mm	0mm
96 hours	90 ± 4 mm	30 ± 3mm
144 hours	90 ± 4 mm	77 ± 3mm

The strong aroma of the essential oils can affect the organoleptic properties of food but the synergistic combinations of essential oils with each other or with other hurdle techniques can reduce this effect. Essential oils represent natural, effective, and consumer-accepted tool against food spoilage causing fungi.

In our study antifungal activity of essential oil of Ajwain (*Trachyspermum ammi*) against fruit spoilage causing fungi isolated from naturally infected fruit samples was investigated by the reversed Petri plate method using Sabouraud dextrose agar medium. To prove the antifungal activity of the essential oil vapours, the spoilage fungi were inoculated on white bread slices in a closed Petri plate system modelling an active packaging.

MATERIALS AND METHODS

Materials

Essential oil

The essential oil of Ajwain (*Trachyspermum ammi L*) was procured from Allin Exporters Private Limited, Noida (India) and stored in air-tight sealed, dark glass bottle at room temperature for further use.

Culture media

In the current study Sabouraud dextrose agar was employed as a growth medium for fungal strains. Media was obtained from HiMedia Laboratories Pvt. Ltd., India.

Molds and culture conditions

Molds were obtained from samples of Tomato (*Solanum lycopersicum*), Orange (*Citrus sinensis*), and Papaya (*Carica papaya*) which were naturally infected with fungi and projected moldy growth on the surface.

Modelling system

White bread was purchased from a local market. Sliced bread was used as a modelling system for testing the efficiency of vapours of essential oil of Ajwain (*Trachyspermum ammi*) against the fungal isolates.

Methods

Isolation

The saline suspensions of the molds from fruit samples were prepared and isolated onto sterile Sabouraud dextrose agar plate. An isolate was selected and the pure culture used for as test organism in the current study.

Antifungal assays

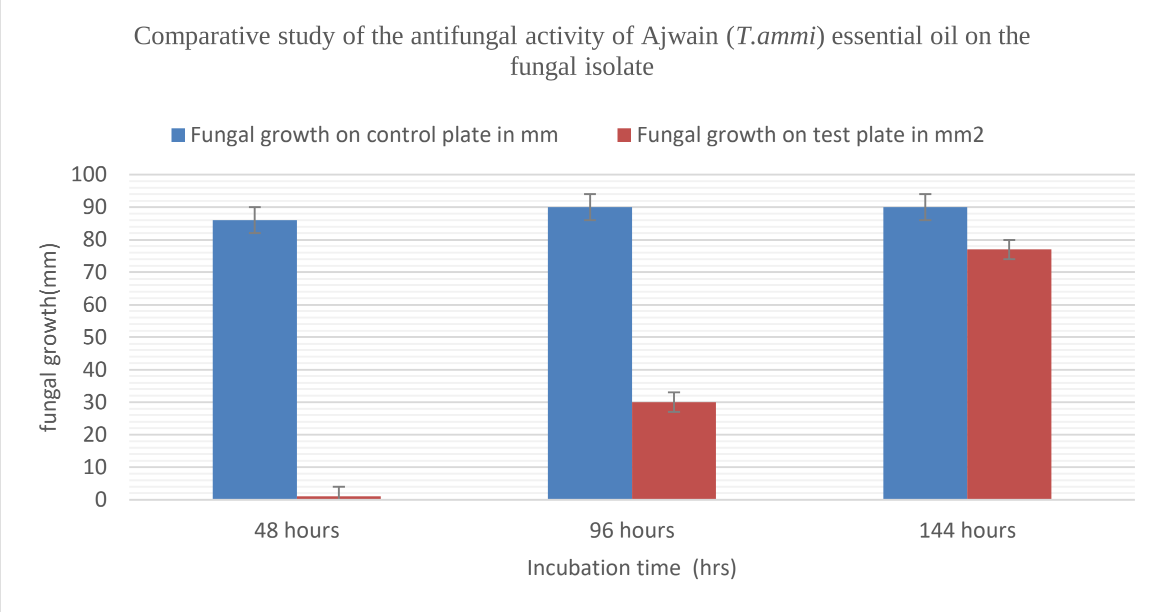
Antifungal activity tests by reversed Petri plate method

Sterile Sabouraud dextrose agar plates were swabbed with the fungal isolates. A sterile paper disc (10mm diameter) was dipped in Ajwain (*Trachyspermum ammi*) essential oil and it was centrally placed on the lid of the plate. Controls were prepared by dipping a sterile paper disc in sterile distilled water and then placed centrally on the lid. Petri plates were sealed and incubated in reversed position for 2 days at room temperature. The plates were observed for colony growth and the zone of inhibition was recorded. The experiment was performed in triplicates and the results were recorded.

Effect of Ajwain (Trachyspermum ammi) essential oil vapours on the shelf life of bread slices

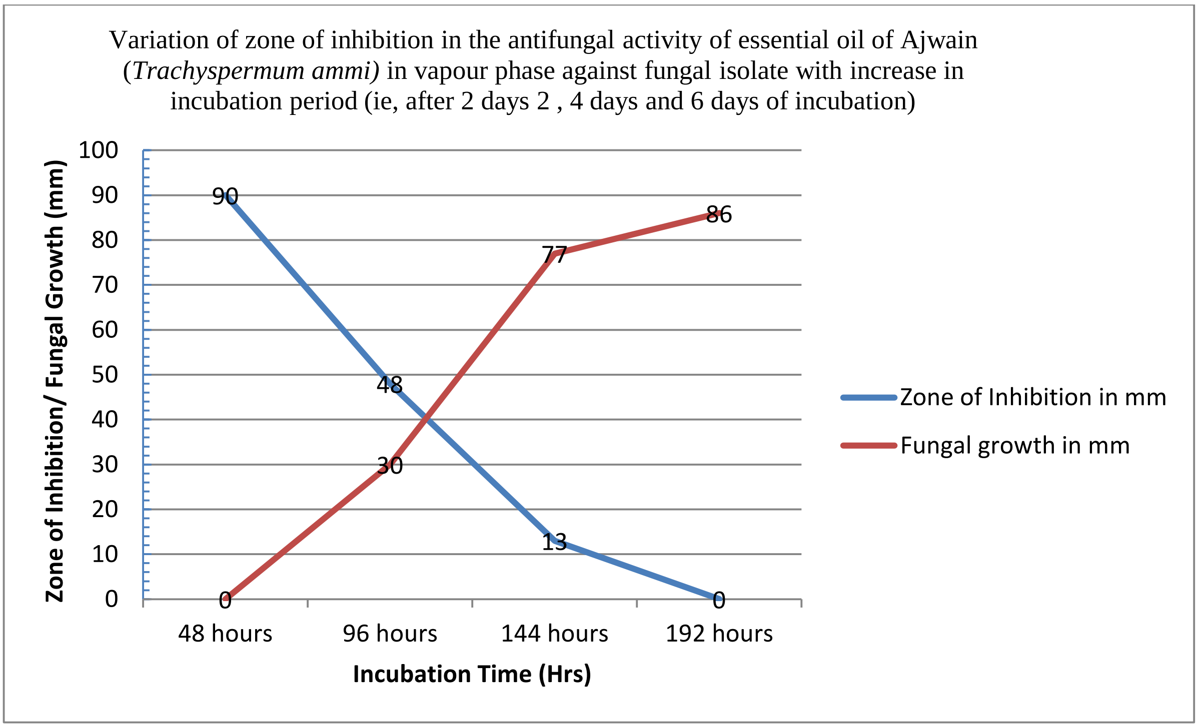
Bread slices were aseptically punched out with a circular mould of 90mm diameter and were placed in sterile Petri plates. Bread slices were inoculated with saline suspension of the fungal isolates. Height of the slices was 9-10mm, so free headspace in a plate could be maintained. A sterile paper disc (10mm diameter) was dipped in pure Ajwain (*Trachyspermum ammi*) essential oil and was centrally placed on the lid of the plate. Petri plates were sealed and incubated for 12 days at room temperature. Control plates contained a paper disc dipped in sterile distilled water placed centrally on the lid. Bread slices were monitored for fungal growth[6].

RESULTS AND DISCUSSIONS



Graph 1: Variation of zone of inhibition in the antifungal activity of essential oil of Ajwain (*Trachyspermum ammi)* in vapour phase against fungal isolate with increase in incubation period (i.e., after 2 days 2, 4 days and 6 days of incubation).

Comparison of the fungal growth on the control and plates with essential oil vapour system (test) are as follows:



Graph 2: Comparative study of the antifungal activity of Ajwain (*T.ammi*) essential oil on the fungal isolate.

Isolation of food spoilage fungi

Tomato, Orange and Papaya are few of the fruits which are commonly consumed by the masses. These fruits when infected show prominent fungal growth on their surfaces and these fungi could be easily isolated from the fruit skin. The fungi affected fruit samples were selected and using inoculation needle different colonies were inoculated into the Sabouraud dextrose agar plate, and then incubated at room temperature for 2 to 3 days. After incubation moldy colonies with varying reverse colourations and characteristics were observed. A colony was arbitrarily selected for further study. Selected colony was purified by repeated streaking. The purified fungi was maintained on Sabouraud agar slants at 4°C throughout the study and used as stock culture.

Antifungal activity using Vapour phase method

Numerous studies have documented the antifungal and anti-bacterial effect of plant essential oils[7,8]. However, the

Figure 2A: Inoculated bread slice placed in vapour phase system, day 10.

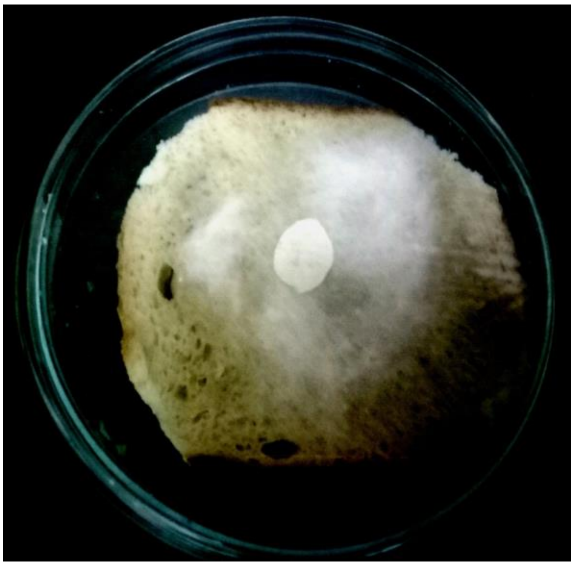


Figure 2B: Inoculated bread slice maintained as control, day 10.

studies have used different methodologies. The extensively used methods are diffusion (from discs, drops or wells) or agar and broth dilution. Solubility and diffusion rates of the oil compounds in the aqueous agar media are of paramount importance, and can lead to misinterpretations in diffusion tests. A third method for testing essential oils is the micro atmosphere method or 'vapour phase method'. This method brings the vapours of essential oils into contact with growth media and micro-organisms. Thus, the problem of inadequate mixing is circumvented, and instead distribution in the air-phase of the active compounds is important. Evaporation inside Petri plates can generate inhibition zones indicating a gradient and non-uniform air-phase distribution of vapour compounds. In the current study, the fungal isolate of was swabbed onto the surface of Sabouraud dextrose agar medium and was subjected to vapours of essential oil of Ajwain (*Trachyspermum ammi*). It was observed that the essential oil vapours significantly inhibited the growth of fungal isolate producing a clear zone of inhibition. The zone of inhibition is attributed to the presence of the active volatile compounds in the essential oil.

It was observed that, for the first 48 hours the test plate showed no growth. However the corresponding control plate showed moldy growth. On the third day slight growth was observed on the circumference of the test plate. This growth showed slight increment in size with every passing day. However the growth rate of the fungi in the test plates was observed to be much slower than that observed in the control plate. The control plate was filled with moldy growth by the third day. Six days after incubation, the zone of inhibition on the test plate was reduced to a minimum (nearly equivalent to the circumference of the disc (Fig. 1A, B & C); (Table. 1); (Graph 1 and 2)

The observations suggest antifungal activity of pure Ajwain (*Trachyspermum ammi*) essential oil. The reduction in the zone of inhibition could possibly be attributed to the consumption and diffusion of the active volatile components of the essential oils.

Effect of essential oil vapours on the shelf life of bread slices

White bread acted as an appropriate modelling system for the fruit spoilage causing isolates. Growth was visible 6 days after inoculation, suggesting the fungi grew slowly on bread. However once the growth was initiated the molds grew well on control plates but particularly poorly on plates with essential oil vapour system. It was observed that the essential oil vapour system delayed colony formation of the molds. Initially on comparison with the control plates no growth was observed on the treated bread slices. Fungal growth decreased significantly on bread slices treated with essential oil vapour systems the results obtained were similar to those in Sabouraud dextrose agar medium. (Fig.2A & 2B).

CONCLUSION

Results of current study indicated that essential oil of *T. ammi* possess antifungal property against strains of fruit spoilage causing fungi. The oil showed prominent antifungal activity against fungi cultured on both synthetic medium as well as bread employed as a modelling system representing food stuffs. It was found that essential oil of *T. ammi* has fungi toxic potential. This finding is supported by the research work carried out by Kamal et al., (2012); Uniyal et al., (2012) and Negero et al., (2014). Therefore, essential oils of T. ammi could be recommended as safe botanical food preservative as it has antifungal activity and presents a potential for a plethora of pharmacological activities. The Ajwain oil being edible posses no threats to humans as such thus, having superiority over synthetic fungicides. In addition, this essential oil has practical applicability as fumigant of food commodities due to their aromatic volatility nature. Moreover, we can also minimize the residual effect of this plant in food commodities by drying food stuffs using sun light before consumption. However, further studies should be conducted to explore the sensory changes caused by vapours of *T. ammi* essential oil and also exploring the efficacy of *T. ammi* essential oils using other toxigenic organism that contaminate food commodities. Solutions can be use of combined treatments, which may provide a scope for more effective synergistic systems.

REFERENCES

1. Victor P. Essential Oils in food production, preservation, flavour and safety. Academic Press, San Diego, 2015, 181-192.
2. Bairwa, R., Sodha, R. S., & Rajawat, B. S. *Trachyspermum ammi*. Pharmacognosy Reviews, 2012, 6(11), 56–60.
3. Gilani, S.R., Mahmood, Z., Hussain, M. Preliminary evaluation of antimicrobial activity of cream formulated with essential oil of *Trachyspermum ammi*. Pak. J. Pharm. Sci. 2013, 26(5), 893-896.
4. Uniyal, V., Bhatt, R.P., Saxena, S.R., Talwar, A. Antifungal activity of essential oils and their volatile components against respiratory tract pathogens causing Aspergilloma and Aspergillosis by gaseous contact. J.Appl.Nat.Sci. 2012, 4, 65-70.
5. Krisch, J., Rentskenhand, T., Horvath, G., Vagvolgyi, C. Activity of essential oils in vapor phase against bread spoilage fungi. Acta Biol Szeged. 2013, 57(1):9-12.
6. Bansod, S. and Rai, M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *Aspergillus niger*. World Journal of Medical Sciences. 2008, 3(2): 81-88.
7. Tyagi, A. K., & Malik, A. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of *cymbopogon citratus*. BMC Complementary and Alternative Medicine, 2010, 10: 65.
8. Kamal, J., Nisha, D.T., Narender, T., Sunil, S., Lalit, T.R. *Tachyspermum ammi* (Ajwain): A Comprehensive Review. IRJP, 2012, 3(5).
9. Hyldgaard, M., Mygind, T., & Meyer, R. L. Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components. Frontiers in Microbiology, 2012, 3:12.
10. Negero G., Woldeamanuel, Y., Asrat, D., and Debella, A. Effect of *Cymbopogon martinii, Foeniculum vulgare*, and *Trachyspermum ammi* essential oils on the growth and mycotoxins production by Aspergillus species. International Journal of Food Science, 2014, 874135.