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## Role of ajwain (*Trachyspermum ammi*) as a feed additives in fish culture

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### Abstract

Ajwain (*Trachyspermum ammi*) from the *Apiaceae* family is distributed throughout India and is mostly cultivated in Gujarat and Rajasthan. Ajwain exhibits several biological effects including analgesic, anti-inflammatory, anxiolytic and antispasmodic activities. The major components of the plant extract are thymol, terpinene, phlandrene, pinene group, myrcene, and cymene which are mostly of oxygenated monoterpenes. Its thymol is known as a major chemical compound and as an antibacterial and antifungal drug. Ajwain is rich in vitamins and minerals; it is also concentrated in health-promoting phytonutrients such as carotenoids ( $\beta$ carotene and lutein) and flavonoids which provides powerful antioxidant protection. Ajwain oil has also been reported to possess to a broad spectrum of fungi toxic behavior against all tested fungi. The present study indicated that the role of Ajwain (*Trachyspermum ammi*) as a feed additives in aquaculture.

**Keywords:** Ajwain, antioxidant, antibacterial, antifungal

### Introduction

Nutrition and health management play a key role for a successful aquaculture. Intensive exploitation of capture fishery resources has lead to almost stagnation in its production. There is a wide gap between the capture fish production and the ever increasing demand for fish. Hence, aquaculture is considered as one of the better alternative means to meet the demand and potential area to tackle the challenges of food security, economy and employment.

Antibiotics and chemotherapeutics are common agents used to handle the outbreak of those diseases in aquaculture. However, the application of these prophylactics leads to the emergence of antimicrobial resistant bacteria and adverse impacts on the water environment. (Done *et al.*, 2015) [6]. Supplementation of natural prophylactics is considered as promising preventive practice which assists in maintaining fish welfare, and a healthy environment (Bruce, Brown, 2017; Guardiola *et al.*, 2018) [4, 8]. Among them, medicinal plants have been considered as promising one. Plant products have been widely applied in aquaculture to enhance growth performance, immune system and to provide antioxidant effects due to their biological compounds such as alkaloids, terpenoids, saponins and flavonoid elements (Reverter *et al.*, 2017) [24].

Moreover, dietary inclusion of plant products can reduce the risks associated with antibiotics and chemotherapeutic and be considered as one of the most effective means for diseases resistance prevention in aquaculture (Nayak, 2010) [22]. Thus, there is a rising trend in use of natural products in recent decades with a focus on medicinal plants as an alternative to antibiotics.

Ajwain belongs to the *Apiaceae* family. The plant is herbaceous, aromatic, without trichome, with erect stems, 20–50 cm height, and with 6–8 split umbels. The ajwain fruit is small, egg-shaped, with dark yellow colour, and smelling like aroma of thymol, which is consumed in dried and ripened form. Its fruit forms the medicinal organ of this plant (Hedge and Lamond, 1987) [9]. The major components of the plant extract are thymol, terpinene, phlandrene, pinene group, myrcene, and cymene which are mostly of oxygenated monoterpenes (Nagulakshmi *et al.*, 2000) [21]. Its thymol is known as a major chemical compound and as an antibacterial and antifungal drug. Ethanol and acetone in this plant extract are effective against most bacteria such as *Pseudomonas*, *Escherichia coli*, *klebsiella*, and *Staphylococcus aureus* (Shankaracharya *et al.*, 2000; Usha *et al.*, 2012) [21, 28, 33].

The addition of thymol as one of the main chemical compositions of ajwain in the diet of rainbow trout in the juvenile phase improves some parameters (Ahmadifar *et al.*, 2011) [1].

Ajwain (*Trachyspermum ammi*) from the *Apiaceae* family exhibits several biological effects including analgesic, anti-inflammatory, anxiolytic and antispasmodic activities. Its seed has been widely consumed as a food flavoring agent and spice. Moreover, the seeds have several therapeutic effects and have been used for treatments of indigestion, flatulence, colic, diarrhea, and dyspepsia. Total essential oil of ajwain has large amounts of carvacrol or thymol. These phenolic compounds are expressed to be either bactericidal or bacteriostatic agents depending on the concentration (Zarshenas *et al.*, 2014) [34]

## Biological properties

### Antibacterial

Ethanol and acetone extract of ajwain seeds possessed an antibacterial activity against two Gram negative food spoilage bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The in vitro antibacterial activity was performed by disc diffusion method. Ethanol extract of ajwain seeds possessed highest activity against *P. aeruginosa* whereas acetone extract exhibited highest activity against *E. coli* (Masih *et al.*, 2012) [33]. Methanol extract of ajwain seeds showed significant antibacterial activity against various strains of bacteria like *P. aeruginosa*, *Bacillus pumilus*, *Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumonia* and *Bordetella bronchiseptica* (Shahidi, 2004) [29].

### Antioxidant

Ajwain is rich in vitamins and minerals; it is also concentrated in health-promoting phytonutrients such as carotenoids ( $\beta$ -carotene and lutein) and flavonoids which provides powerful antioxidant protection. Antioxidant potential of blend of ajwain seed extract was evaluated by two different in vitro assays; the hydrogen peroxide ( $H_2O_2$ ) scavenging assay and nitric oxide radical scavenging assay. Results revealed that ajwain extract exhibited significant effect in inhibiting hydrogen peroxide reaching up to 70.04% at concentration of 200  $\mu$ g/ml. Antioxidant activity in the extract of fresh and frozen ajwain leaves was subjected to in vitro DPPH free radical scavenging assay. Results showed that frozen ajwain leaves exhibited significantly higher antioxidant activity than fresh one (Mazahir *et al.*, 2015) [16].

### Antifungal

Ethanol extract of ajwain seeds showed antifungal activity against selected fungi (*Aspergillus flavus*, *A. ochraceus*, *A. niger*, *A. oryzae*, *Fusarium moniliforme*, *Penicillium sp.*) using agar well diffusion assay (Odhav *et al.*, 2002) [23]. Ajwain seed essential oil also exhibited a broad spectrum of fungitoxic behavior against *A. niger*, *A. flavus*, *A. oryzae*, *A. ochraceus*, *F. moniliforme*, *F. graminearum*, *P. citrium*, *P. viridicatum*, *P. madriti* and *Cheilomenes lunata* and absolute mycelial zone inhibition was obtained at a 6  $\mu$ l dose of the oil (Thangam and Dhananjayan, 2003) [31]. Ajwain seeds inhibited the growth of all test fungi by 72-90%. Fungicidal effect of ajwain seed essential oil on *A. Niger* and *Cochlostyla ovoidea* was also evaluated and minimum inhibitory concentration was observed at 5000 $\mu$ g/ml (Dwivedi and Singh, 1998) [7].

### Antimicrobial

Ajwain seed oil showed antimicrobial activity on fifty-five bacterial strains with minimum inhibitory concentration (MIC) < 2% (v/v) except against *P. aeruginosa* (Mayaud *et al.*, 2008) [15]. Diethyl ether fraction of ajwain exhibited good antibacterial and antifungal activity against multi drug resistant (MDR) strains of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, *E. coli* and reference strains of *Streptococcus mutans* and *S. bovis* (Khan *et al.*, 2010) [12]. The active compounds, responsible for the antimicrobial activity of ajwain were carvacol and thymol (Saxena and Vyas, 1986) [27]. Antimicrobial activity of essential oil of ajwain (*Trachyspermum ammi* L.) was found susceptible to both gram-positive and gram-negative bacteria (Upadhyay *et al.*, 2010) [32]. Ajwain oil has also been reported to possess to a broad spectrum of fungi toxic behavior against all tested fungi. Javed *et al.* (2012) [10] also reported that the antibacterial and antifungal activity of aqueous and methanolic extract of *T. ammi* against dominant strains of bacteria such as *Pseudomonas syringica*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus sp* and fungi such as *Aspergillus flavus*, *A. niger* and *Candida albicans* (Javed *et al.*, 2012) [10].

### Anthelmintic

Anthelmintic activity in ajwain was exerted by interference with the energy metabolism of parasites through potentiation of ATPase activity and thus loss of energy occurred. Anthelmintic activity of ajwain, showed its effect against specific helminths, e.g. *Ascaris lumbricoides* in humans and *Haemonchus contortus* in sheep. The plant was also reported to possess cholinergic activity with peristaltic movements of the gut, thus helped in expulsion of intestinal parasites which might also be a contributory factor to its anthelmintic activity (Tamura and Iwamoto, 2004; Jabbar *et al.*, 2006) [30, 11]

### Ajwain acts as feed additive

Mohsen Ali *et al.* (2017) [18] conducted a study to investigate the effect of vitamin E and selenium, marjoram (*Origanum spp.*) and ajwain (*Trachyspermum ammi*) extracts on growth performance and lysozyme activity of rainbow trout fingerlings. Results revealed that the addition of ajwain and marjoram extracts at 1–2% could significantly increase ( $p < .05$ ) some growth factors, including BWI, SGR, FCR, LG and CF as well as reduce economic conversion ratio (ECR) in rainbow trout after active feeding.

Mohsen Ali *et al.* (2017) [18] evaluated that the effects of vitamin E and selenium, marjoram (*Origanum spp.*) and ajwain (*Trachyspermum ammi*) extracts on Growth performance and lysozyme activity of rainbow trout fingerlings. The SR was 100% after applying the oxygen shock test on rainbow trout larvae fed with 1% and 0.5% ajwain extract, which was significantly higher than other groups ( $p < .05$ ). However, all the mentioned supplements showed a greater SR compared to the control group (60%). Similarly the effect of adding 2% garlic plant to the diets of rainbow trout led to elevate SR (Gabor *et al.*, 2010). The use of parsley (*Petroselinum sativum*) increased the SR of the common carp (Mooraki *et al.*, 2014) [20]. The results of all these studies are similar to the findings of the present study, indicating an increase in the survival rate of baby rainbow trout fed with the marjoram and ajwain extracts.

Mohsen Ali *et al.* (2017) <sup>[18]</sup> conducted that the effects of vitamin E and selenium, marjoram (*Origanum* spp.) and ajwain (*Trachyspermum ammi*) extracts on Growth performance and lysozyme activity of rainbow trout fingerlings. Results revealed that lysozyme demonstrated that fish fed with diets containing 1% ajwain and marjoram levels showed significantly increased levels of the lysozyme compared with the control group. Few studies demonstrated that lysozyme activity was induced by black cumin in rainbow trout (Celik *et al.*, 2017) <sup>[5]</sup> and dill (*Anethum graveolens*) and garden cress (*Lepidium sativum*) in common carp (*Cyprinus carpio*) (Bilen *et al.*, 2018) <sup>[3]</sup>.

The addition of 0.2–0.3 g of thymol as one of the major compositions of ajwain in the diet of rainbow trout in the juvenile phase improved growth performance (Ahmadifar *et al.*, 2011) <sup>[1]</sup>. The application of 3% ajwain seed extract gave the best antioxidative and antimicrobial activities, as well as sensory scores up to 15 days of storage followed by 3% shallot fruit extract in rainbow trout (Sara Raeisi *et al.*, 2016) <sup>[26]</sup>.

Safari *et al.* (2019) <sup>[25]</sup> revealed that the growth performance was improved by the supplemented *Ferula asafetida* powder at the rate of 20–25 g/kg diet in koi carp. Moreover, the application of 0.2% soaked seed of ajwain could improve nutrient absorption, enhance gut micro-flora and increase digestibility as an alternate to antibiotic growth promoters in diet of broiler Matheus *et al.* (2019) <sup>[14]</sup> demonstrated that the Nile tilapia fed with diets containing 400, 800 and 1200 mg vegetable choline/kg feed resulted an augmentation on live weight, weight gain and feed intake after 60 days. Better growth performance and body composition were recorded in the diet fed with 5 and 10g rosemary leaf powder /kg diet in Nile tilapia (Mohammed *et al.*, 2019) <sup>[19]</sup>. Whereas, weight gain, specific growth rate and feed conversion ratio were improved by dietary lemon verbena at 10–20 mg/kg diet in Siberian sturgeon (Milad *et al.*, 2021) <sup>[17]</sup>.

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