

EVALUATION OF PHYTOCHEMICALS IN CERTAIN INDIAN SPICES FOR QUORUM SENSING INACTIVATION IN A PLANT PATHOGENIC BACTERIA

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ABSTRACT

Quorum sensing is a mechanism where bacteria produce and release chemical signals in search of similar cells in their close surroundings. Quorum sensing (QS) is a key regulator of virulence factor expression which includes protease, pyoverdinin, pyocyanin etc in *Pseudomonas* spp. Present study was made to evaluate effect of aqueous extracts of six Indian spices *Allium sativum*, *Trigonella foenum*, *Trachyspermum amoni*, *Brassica juncea*, *Syzygium aromaticum* and *Piper nigrum* for their anti-quorum sensing activity against *Pseudomonas* spp. a lab isolate. Of all the six spices tested *Allium sativum*, *Piper nigrum* and *Syzygium aromaticum* showed significant inhibition in quorum sensing activity with less effect on bacterial growth. Our findings suggest that the quorum quenching mechanisms are not related to bacteriostatic or cidal effects.

INTRODUCTION

Quorum sensing is a mechanism where bacteria produce and release chemical signals. As environmental conditions often change rapidly, bacteria need to respond quickly in order to survive. These responses include adaptation to availability of nutrients, defense against other microorganisms, which may compete for the same nutrients and the avoidance of toxic compounds potentially dangerous for the bacteria. It is very important for pathogenic bacteria during infection of a host to co-ordinate their virulence in order to be able to establish successful infection. (Lian-Hui zhang and Yi-Hu Dong, 2004). Several chemicals and enzymes have been identified in recent years (Allison *et al.*, 2008) that target the key components of bacterial quorum-sensing systems. These quorum-quenching reagents highlight the feasibility of preventing bacterial infections by blocking bacterial cell-cell communications (Yi-Hu Dong and Lian-Hui, 2005).

Recently Quorum-quenching substances were found in terrestrial plants *Pisum sativum* (pea) and *coronilla varia* (crown vetch) (Rasmussen *et al.*, 2005). Although the chemical nature of the inhibitory substances and their effect on bacterial virulence are not clear, this finding is interesting because it shows that quorum quenching could also be one of the intrinsic plant defense mechanisms against pathogenic invaders (Max Tepliski *et al.*, 2000). Based on such observations in this study, attempt was made to evaluate the crude extracts of certain Indian food spices which were known to possess medicinal values.

MATERIALS AND METHODS

Pseudomonas sp. isolated from infected tomato leaf was cultured and maintained in nutrient broth and nutrient agar media.

Preparation of crude extract of spices: Aqueous extracts of the Indian spices Garlic (*Allium sativum*), Fenu greek (*Trigonella foenum-graecum*), Bayleaf (*Trachyspermum amoni*), Mustard (*Brassica juncea*), Cloves (*Syzygium aromaticum*) and Pepper (*Piper nigrum*) were prepared (1-2 g in 5-10 mL). Serial dilutions were made and used to check for quorum quenching activity.

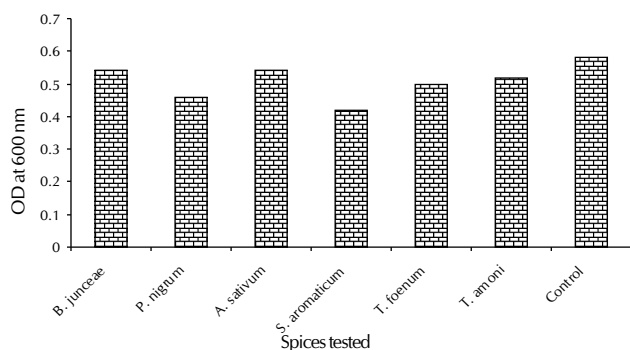
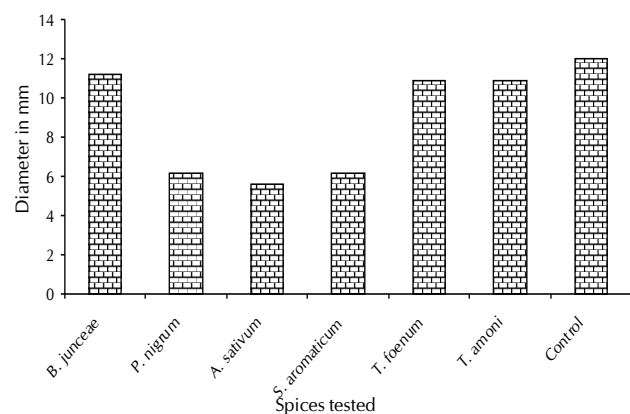
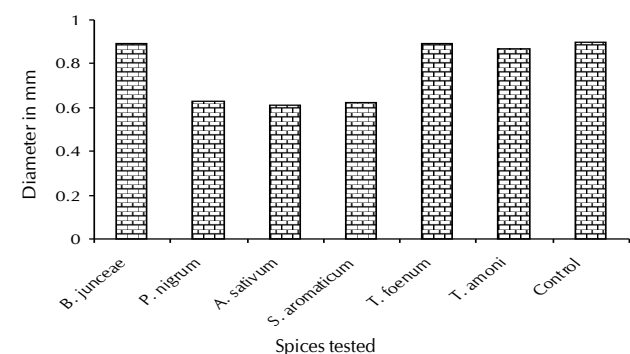
Quorum quenching activity of crude extracts against *Pseudomonas* sp.: Serial dilutions of the crude extract were made in nutrient broth and 0.1 mL of overnight grown culture of *Pseudomonas* sp. was added and incubated overnight at 37°C. After incubation, the growth was monitored by recording OD at 600nm. Since the production of protease, pyocyanin were quorum sensing dependent these parameters were monitored in the treated samples for all dilutions. Pyocyanin assay was done as described by Essar *et al.*, 1990 and protease activity was done using skim milk agar as described by Brown.

RESULTS AND DISCUSSION

Quorum sensing inhibitors (QSI) were isolated from natural sources such as plants and fungi. Plants have coexisted with QS bacteria for millions of years. It is expected that at least some of them produce QSI compounds in order to reduce the

Table 1: Therapeutic values of different Indian spices

Common name	Botanical name	Commercial part	Therapeutic value
Garlic	<i>Allium sativum</i>	Bulb	Anti-bacterial, Fungicidal and Insecticidal
Clove	<i>Syzygium aromaticum</i>	Flower bud	Used in Ayurveda, Chinese medicine, carminative, anti helminthic
Fenugreek	<i>Trigonella foenum-graecum</i>	Fruit	Colicflatulence, dysentery, diarrhea, dyspepsia, rickets, gout and diabetes
Bayleaf	<i>Trachyspermum amoni</i>	fruit	Anti microbial, antifungal, hypoglycemic, anti ulcerogenic
Mustard	<i>Brassica juncea</i>	seed	Flavoring agent, preservative, antioxidant
pepper	<i>Piper nigrum</i>	Dried fruit	Constipation, Diarrhea, gangrene, heart disease, insomnia, liver and lung problems, sunburn, tooth decay etc

**Figure 1: Effect of spice extracts on Pseudomonas growth****Figure 2: Effect of spice extracts on protease activity in Pseudomonas spp****Figure 3: Effect of spice extracts on pyocyanin activity in Pseudomonas spp**

pathogenic capability of colonizing, competing and infective bacteria. Plants like Crown vetch, Carrot, Soybean, Waterlily, Tomato, Pea seedlings, Chilli and Garlic have been found to produce compounds capable of interfering with bacterial QS (Rasmussen *et al.*, 2005). In this context crude aqueous extracts of Indian spices were evaluated for their Quorum quenching activity. All these spices were reported with good

therapeutical value and often used in Ayurveda and Unani medicine (Table 1).

From the data obtained, it is evident that *Piper nigrum*, *Allium sativum* and *Syzygium aromaticum* could drastically reduce the virulence factors i.e. protease production by 50% when compared with control (Fig. 2). The same spices also decreased Pyocyanin production by 30% when compared to control (Fig. 3). Interesting feature of these extracts was they did not effect growth of *Pseudomonas* spp. (Fig. 1). With other spices, *Trigonella foenum*, *Trachyspermum amoni*, *Brassica juncea*, growth was reduced when compared to control but doesn't have an effect on protease and pyocyanin production. The phytochemicals present in *Piper nigrum*, *Allium sativum* and *Syzygium aromaticum* were able to reduce the virulence factor production which could be playing a role in QSI. Similar result with garlic was reported by Rasmussen *et al.* (2005) which states that QSI from garlic exerts a strong antagonistic effect on Lux-R based QS but interestingly has no effect against *P. aeruginosa* QS. However our study with *Pseudomonas* sp, plant pathogen showed inhibition of protease and pyocyanin which are QS controlled.

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