

Rhizosphere effect in seed spices plants grown under semi-arid conditions

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Abstract

An experiment was conducted to assay the Rhizosphere effect in seed spices plants grown under semi-arid conditions. Rhizospheric Soil samples of Ajwain (*Trachyspermum ammi*), anise (*Pimpinella anisum*), celery (*Apium graveolens*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), fenugreek (*Trigonella foenum-graecum*) and nigella (*Nigella sativa*) were used for estimation of culturable microbial population. Microbial population in rhizospheric soil and bulk soil was compared for rhizosphere effect in selected seed spices plants. The rhizosphere effect is exemplified by the observation that total culturable bacterial population in the rhizosphere are about 4 to 1.5 fold higher compared to the numbers in bulk soil for majority of seed spices except cumin and nigella. The microbial activity in terms of dehydrogenase enzyme was also estimated in the rhizospheric soil and bulk soil of each seed spices plant. Among the Apiaceae family highest dehydrogenase activity was recorded in ajwain soil samples, 4.26 and 4.53 $\mu\text{g TPF g}^{-1}$ for bulk and rhizosphere soil, respectively. The lowest bulk (non-rhizosphere) dehydrogenase activity ($1.17 \mu\text{g TPF g}^{-1}$) was found in cumin followed by rhizosphere soil of anise ($1.2 \mu\text{g TPF g}^{-1}$).

Key words : Dehydrogenase, microbial population, rhizosphere effect, seed spices.

Introduction

Seed spices are group of annual herbaceous plants which are cultivated for their dry fruits or seeds mainly used as spice. The major seed spices are cumin, coriander, fenugreek, fennel, ajwain, nigella, dill, celery, anise and caraway. India is the leading country in production, consumption and export of these seed spices. Rajasthan and Gujarat state of India together contribute more than 80 percent of the total national seed spices production. These are mainly *rabi* (winter) season crops and grow well in semi-arid and rainfed conditions of Nagaur, Pali, Sirohi, Jaisalmer, Kota and Baran districts of Rajasthan. These crops are also grown more or less by other states like Madhya Pradesh, Orissa, Tamil Nadu, Andhra Pradesh, Karnataka, Bihar, Uttar Pradesh, Punjab and West Bengal. About 1.45 millions tonnes of seed spices were produced in India from an area of 1.74 million hectare with a productivity of 833 kg ha^{-1} during 2016-17. These seed spices are comprise of species /genus from three families of Angiospermic plant of Eudictos. Ajwain (*Trachyspermum ammi*), anise (*Pimpinella anisum*), celery (*Apium graveolens*), coriander (*Coriandrum sativum*) and cumin (*Cuminum cyminum*) belong to apiaceae while fenugreek (*Trigonella foenum-graecum*) belongs to fabaceae and nigella (*Nigella sativa*) belongs to ranunculaceae.

During seed germination and seedling growth, the developing plant interacts with a range of microorganisms present in the surrounding soil. As seeds germinate and roots grow through the soil, the release of organic material provides the driving force for the development of active microbial populations in a zone that includes plant root and surrounding soil in a few millimeter of thickness. This phenomenon is referred as the rhizosphere effect (Morgan and Whipps, 2001). "Rhizosphere effect", a term defined by collective processes occurring at the root-soil interface of a plant and includes root exudation, microbial activity, genetic exchange, nutrient transformation and gradient diffusion. In living plants, organic carbon released by plant roots is decomposed to CO_2 in a mechanism known as rhizosphere priming effect. Although the estimate of plant carbon economy is still controversial, approximately one-third to half of total assimilated carbon is allocated to below-ground, of which 15-25% is exuded from the roots into the soil to induce fast carbon-turnover in the rhizosphere (Kuzyakov, 2002). Due to intensive carbon uptake by the roots, other nutrients in the rhizosphere are strongly limited. In contrast, in a root-free soil, all the nutrients except carbon are unlimited. The surplus of easily available carbon and strong nutrient limitation, together, make the rhizosphere milieu strongly different from that of the root-free zones. The increased microbial number and activity in the rhizosphere compared to those in bulk

soil are mainly due to the release of organic carbon by the plant roots (Hartmann *et al.*, 2009). Rhizosphere, the soil adjacent to plant-roots, is a unique niche for microbial colonization. It is a complex chemical matrix replete with diverse microbial species. Rhizosphere microbial community is recruited from the adjoining soil which acts as a microbial seed bank, while the plants determine which members of this bulk soil pool of microorganisms will flourish and thrive in the rhizosphere (Lennon and Jones, 2011). Microbiological profile of coriander (Mishra *et al.*, 2013), fennel (Mishra *et al.*, 2015) and isabgol (Mishra *et al.*, 2016) crop rhizosphere has been reported from the soils of major productions regions of Rajasthan. Rhizosphere microbial populations are not simple listeners. They too communicate among themselves in addition to interacting with the plant root system. Based on their primary effects, the beneficial rhizosphere organisms are generally classified into two broad groups (i) biological control agents that indirectly assist with plant productivity through the control of plant pathogens and (ii) plant growth promoting microorganisms with direct effects on plant growth promotion. However, in present investigation efforts were made for appraisal of rhizosphere effect on seven seed spice crops that were grown at Ajmer which falls under semi-arid region of Rajasthan, India.

Material and methods

Rhizospheric Soil samples of Ajwain (*Trachyspermum ammi*), anise (*Pimpinella anisum*), celery (*Apium graveolens*), coriander (*Coriandrum sativum*) and cumin (*Cuminum cyminum*) fenugreek (*Trigonella foenum-graecum*) and nigella (*Nigella sativa*) were collected at flowering stage of individual seed spices from the experimental farm of ICAR-NRCSS, Tabiji, Ajmer (Raj.) during 2015-16. The non-rhizospheric soil sample upto depth of 10-12 cm for each crop was taken as bulk soil separately. The culturable mesophilic microbial count was done through serial dilution techniques and spread plating of 0.1ml from selected dilutions on nutrient agar medium and potato dextrose agar medium (Hi-media). The inoculated Petriplates were incubated at 25° C for 3-5 days and colony forming units were counted for analysis of microbial population of bacteria and fungi separately. The dehydrogenase activity of rhizospheric and non-rhizospheric soils of different seed spice crops grown at ICAR-NRCSS, Ajmer experimental field was estimated by method of Casida *et al.*, (2000) using 2,3,5-Triphenyltetrazolium chloride (TTC) as a substrate for dehydrogenase enzyme activity in the soil samples. The Dehydrogenase activity was expressed as µg TPF produced per g of soil.

Results and discussion

To illustrate the rhizosphere effect we compared rhizosphere bacterial and fungal communities of selected seven seed spices. Total bacterial count in bulk soil and in the rhizosphere of major seed spices crops are presented in table1. The rhizosphere effect is exemplified by the observation that total culturable bacterial population in the rhizosphere are about 4 to 1.5 fold higher compared to the numbers in bulk soil for majority of seed spices except cumin and nigella. The rhizosphere: bulk soil (R: S) ratio of bacterial population ranged from 4 to 0.69 and the maximum R: S ratio was recorded with coriander crop (4:1) followed by fenugreek and minimum was recorded with cumin (0.69:1). The rhizosphere: bulk soil (R: S) ratio of fungal population ranged from 4.2 to 0.83. The highest R: S ratio was observed with fenugreek (4.2:1) followed by coriander (3.8:1) and lowest R:S ratio was observed with nigella (0.83:1) followed by ajwain (1.28:1) (Table 2). Similar observations were reported by Dotaniya and Meena (2015) for the rhizosphere effect on different crops in which ratio of total microbial population of rhizosphere and bulk soil varied from 24 to 3 (R:S ratio). The dehydrogenase activity of rhizospheric and non-rhizospheric soils of different seed spice crops grown at ICAR-NRCSS, Ajmer experimental field was estimated by using 2,3,5-Triphenyltetrazolium chloride (TTC) as a substrate for dehydrogenase enzyme activity in the soil samples. The perusal of data revealed that there is significant difference in dehydrogenase activity of different seed spices soil samples both at species level and family level (Table 3). Among the Apiaceae family highest dehydrogenase activity was recorded in ajwain soil samples, 4.26 and 4.53 µg TPF g⁻¹ for bulk and rhizosphere soil, respectively. The lowest bulk (non-rhizosphere) dehydrogenase activity (1.17 µg TPF g⁻¹) was found in cumin followed by rhizosphere soil of anise (1.2 µg TPF g⁻¹). This may be due to small root system and thereby supporting less microbial population due to lesser root exudates. The fabaceae family seed spices i.e. fenugreek was found to be supportive of large microbial population as evident from higher dehydrogenase activity in rhizosphere (3.40 µg TPF^{h-1} g⁻¹) as well as non-rhizosphere (3.02 µg TPF^{h-1} g⁻¹). In case of ranunculaceae (nigella) seed spice there was higher dehydrogenase activity in non-rhizosphere than rhizosphere. This may be attributed to the root exudates of nigella having antimicrobial properties. Though, there are many reports that seed spices have antimicrobial activity but the chemical compounds responsible for their anti-microbial property many not be excreted in the root exudates as in seeds. It

Table 1. Bacterial population (cfu x10⁷ g⁻¹) in rhizosphere of different seed spices and their R/S ratio

| Seed spice crop | Rhizosphere | Bulk soil | R:S ratio |
|-----------------|-------------|-----------|-----------|
| Coriander | 5.05 | 1.26 | 4.00 |
| Cumin | 4.50 | 6.50 | 0.69 |
| Fennel | 15.60 | 4.20 | 3.71 |
| Fenugreek | 23.50 | 6.25 | 3.76 |
| Ajwain | 13.80 | 7.20 | 1.91 |
| Celery | 36.0 | 12.0 | 3.00 |
| Nigella | 2.30 | 2.47 | 0.93 |
| Mean | 14.39 | 5.69 | 2.57 |
| S D | 11.65 | 3.40 | 1.33 |

Table 2. Fungal population (cfu x10⁶ g⁻¹) in rhizosphere of different seed spices and their R/S ratio

| Seed spice crop | Rhizosphere | Bulk soil | R:S ratio |
|-----------------|-------------|-----------|-----------|
| Coriander | 32.0 | 8.40 | 3.80 |
| Cumin | 3.40 | 1.82 | 1.80 |
| Fennel | 22.4 | 7.8 | 2.87 |
| Fenugreek | 18.20 | 4.31 | 4.20 |
| Ajwain | 12.60 | 9.80 | 1.28 |
| Celery | 19.0 | 5.3 | 3.58 |
| Nigella | 1.17 | 1.40 | 0.83 |
| Mean | 15.53 | 5.54 | 2.62 |
| S D | 10.37 | 3.13 | 1.27 |

Table 3. Dehydrogenase activity (µg TPF^{h-1}g) in seed spices

| Family | Seed Spices | Dehydrogenase activity (µg TPF ^{h-1} g) | |
|---------------|-------------|--|-------------------|
| | | Bulk soil | Rhizospheric soil |
| Apiaceae | Ajwain | 4.26 | 4.53 |
| | Anise | 2.11 | 1.20 |
| | Celery | 2.80 | 2.30 |
| | Coriander | 2.10 | 2.40 |
| | Cumin | 1.17 | 1.37 |
| Leguminoceae | Fenugreek | 3.02 | 3.40 |
| Ranunculaceae | Nigella | 3.90 | 3.48 |
| | SD | 1.08 | 1.20 |
| | Mean | 2.76 | 2.66 |

is generally considered that compared to non-rooted bulk soil, the soil compartment directly around the plant root contains much larger populations of microorganisms, but this may not be always true. Some plant roots exudates are also inhibitory to certain types of microorganisms; thereby reduce the total microbial population under the root zone or rhizosphere.

Rhizosphere, the intersection between soil and plant roots, is a chemically complex environment which ropes the development and growth of diverse microbial communities. The composition of the rhizosphere microbiome is dynamic and controlled by multiple biotic and abiotic factors that include environmental parameters, physiochemical properties of the soil, biological activities of the plants and chemical signals from the plants and bacteria which occupy the soil adherent to root-system (Halder and Sengupta, 2015). The microbial action in the rhizosphere is essential for plant functioning as it assists the plant in nutrient uptake and offers protection against pathogen attack. Microbiological studies in the soil environment are hampered by the fact that the largest proportion of soil bacteria as yet cannot be cultured. However, developments in metagenomics provide a more complete picture of the rhizosphere microbiome. The transcriptomic studies of the microbiome have been initiated to reveal microbial activities in complex environments (Jansson *et al.*, 2012). Unraveling processes that drive selection and activities of the rhizosphere microbiome will open up new avenues to manipulate crop health and yield (Bakker *et al.*, 2013).

The increased microbial numbers and activities in the rhizosphere are due to the release of large amounts of organic carbon by the plant roots (Hartmann *et al.*, 2009). It is well established fact that plant root exudates differ between plants species, differences in rhizosphere microbiomes of different plant species are to be expected. Indeed plant-specific microbial communities could be isolated from roots in studies. More recent studies, in which the rhizosphere microbiomes were characterized based on direct extraction of total community DNA, also provide strong evidence for plant species-specific microbiomes (Kirk *et al.*, 2005). The roots of wheat, maize, rape, and barrel clover were shown to carry different bacterial communities as a consequence of assimilation of root exudates (Haichar *et al.*, 2008). Bacterial community structures in field grown potato rhizospheres were affected by the growth stage of the plant (Inceoglu *et al.*, 2013).

The plant genotype is also a dynamic force for the selection of specific elements from the bulk soil microbial

community. In addition, when stressed, plants seem to actively select specific elements of their bacterial rhizosphere microflora. This is most clearly observed in so-called disease suppressive soils, in which disease will not develop despite the presence of a virulent pathogen and a susceptible plant. Disease suppressiveness is due to microbial activity and usually needs an outbreak of disease to develop.

Conclusion

Among the Apiaceae family highest dehydrogenase activity was recorded in ajwain soil samples both in non-rhizosphere and rhizosphere, respectively where as lowest non- rhizosphere dehydrogenase activity was found in cumin. This may be due to small root system and thereby supporting less microbial population due to lesser root exudates. The fabaceae family seed spices i.e. fenugreek was supporting growth of higher microbial population as evident from higher dehydrogenase activity in rhizosphere. In case of ranunculaceae (nigella) seed spice there was higher dehydrogenase activity in non-rhizosphere than rhizosphere.

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