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Micro-Organisms In Cultivation And Activation Of Antioxidant Compounds In Spices

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Abstract

Microorganisms play a very noble role in spice cultivation, where they promote plant growth and are the most sustainable way to improve overall health and yield. They improve the water and mineral acquisition by modifying the plant's roots. This article discusses the role of plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF), which are commonly used in spices and medicinal herbs to enhance the antioxidant potential in plants to build immunity against pathogens, herbivory, biotic and abiotic stress. This article also highlights the impact of plant growth-promoting microbes (PGPM) in spice crops, in their morphology and physiology, being broad-spectrum and environmentally sustainable.

Keywords: Plant growth-promoting microbes (PGPM), Spice and medicinal crops, Pathogens, Antioxidants.

Introduction

Plant derivatives are rich sources of various bioactive compounds that are of great use for human metabolism, one of the richest sources of these compounds is spices (Toussaint *et al.*2007; Kandari *et al.* 2012). Spices aren’t just limited to providing essential bio-enzymes; they have their role in the kitchen in enhancing the flavor and aroma of the food to serve as medicines with their anti-microbial, anti-inflammatory and anti-oxidizing enzymes. The growing demand due to the enormous population growth has led to the use of inorganic fertilizers and pesticides to increase production, which has reduced the quality of produce and biomagnification. This problem can be dealt with through the use of microorganisms in spice cultivation. The plant growth-promoting microbes (PGPMs) are microbial colonies that grow in specialized ecological niches surrounding the roots of the plants. They help them in nutrient absorption, protect against soil-borne microbes, promote antioxidant production, which is a vital plant metabolite, a capable scavenger of oxygen-free radicals and antiproliferative properties to fight against biotic and abiotic stress (Wink and Schimmer 2010).

Plant Growth Promoting Microbes

The rhizosphere of a plant is a large deposit of plant growth-promoting microbes (PGPMs), mainly bacteria and fungi, which help the plant grow with vigor and enhance its innate immunity to fight against pathogens.

Role of Rhizobacteria

Rhizobacteria play a crucial role in promoting seed germination, increasing yield, the procurement of nutrients and resistance against biotic and abiotic stresses. These rhizobacteria modify plant physiological and metabolic processes by helping in nitrogen fixation, providing the required amount of nitrogen to the plant to improve its vegetative growth. The rhizobacteria mostly belong to the following genera: Azotobacter, Azospirillium, Bacillus, Cellulomonas, Clostridium, Enterobacter, Flavobacterium, Micrococcus, Pseudomonas, Rhizobium, Sinorhizobium, etc. are responsible for enhancing plant growth and productivity (Rajasekar and Elango 2011).

Rhizobacteria activate many phytochemicals, which provide the plant defense power to protect the crop against viral soil-borne diseases, bacterial, fungal, and nematode infections in the plant. This is done by a different mechanism of antibiotic action, phytohormones, lytic enzymes, antioxidants and nutrient composition. Microbes also increase the availability of phosphate for spice crops and protect and plant pests and pathogens (Bafana and Lohiya 2013).

The interaction of the microbe and the plant provides the spice crops with both induced resistance and innate resistance. In recent days, the use of rhizobacteria has been used as biofertilizers to cultivate spices with minimal to no use of pesticides and to get improved yield without destroying the bioactive components of the spices.

Role of Rhizosphere Fungi

Rhizosphere fungi grow near the root zone and flourish there as saprophytes, being non-pathogenic, belonging to the fungi family of ascomycetes, basidiomycetes and oomycetes (Hossain *et al*. 2017). These are also called Plant Growth-Promoting Fungi (PGPF), they promote plant vigour, nutrient uptake, flowering, photosynthetic cycle and activate antioxidants (Murali *et al.* 2013; Muslim *et al*. 2017). PGPFs are vital in inducing the secretion of defense-related enzymes like phenylalanine ammonia lyase, peroxidase, chitinase, etc. and metabolites like lignin, callose and phenols. Though they are saprophytes, they still help the crop in inducing antioxidants, which are necessary for the defense mechanism of the plant.

Importance of Plant Growth Promoting Microbes

1. The antioxidants and bio-enzymes secreted by different parts of the crop, i.e., leaf, flower bud, stem, bark, seeds, etc., are beneficial for the plant to face the pathogens and are requirements of the human diet.
2. The bio-enzymes having anti-microbial, anti-cancerous, anti-inflammatory, anti-diabetic, neuroprotective, etc., increase their value both as nutrition and as a product for sale in the market.
3. Plant growth-promoting microbes are necessary constituents in the production of spices, as they help in inducing the enzymes to their maximum potential to bring aroma to them.
4. Micro-organisms decompose organic matter in the soil, releasing nutrients and continuing the nutrient cycle
5. These organisms improve the soil structure by breaking down the nutrients in the soil into forms that the plants can readily absorb.
6. Provides tolerance against abiotic stress like drought, salinity, temperature fluctuation, etc.

Application Methods

Seed Treatment: Application of the microorganisms on the seed before sowing to promote healthy germination and seed growth.

Soil Application: Apply micro-organisms to the soil during planting or as a foliar spray to promote healthy growth in plants

Foliar spray: Apply micro-organisms as a foliar spray for pathogen control.

Types of Microbes Based on Their Presence

Extracellular Plant growth-promoting rhizobacteria: located on the rhizoplane or in spaces between the cells of the root cortex. Examples: Agrobacterium, Azotobacter, Azospirillium, Bacillus, Pseudomonas, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, and Serratia

Intra-cellular plant growth-promoting rhizobacteria: present inside cells of the root nodule: Example: Allorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium and Frankia species.

Endophytic Fungi: These colonize inside the host plant parts to exchange metabolites. E.g., Aspergillus, Fusarium, Penicillium, Phoma and Trichoderma, Botrytis, etc.

Epiphytic Fungi: These reside on the plant surface and remain in contact with the outer environment. E.g., Xylaria, Tirisporella, Sordaria, Venturia, Pyricularia, Pleospora, Phyllospora, Monilinia, Melanospora, Hypocrea, Helminthosphaeria, Elsinoe, Alternaria alternate, Cladosporium

Table 1. Types of microbes based on functions

Type of microbe	**Species**	**Mode of action**
Bacteria	*Rhizobium* *Azospirillium,* *Bacillus subtilis*	Nitrogen fixation in the soil, enriches soil fertility for spice plants. Enhances nutrient availability, particularly nitrogen, and promotes plant growth through biological nitrogen fixation. Acts as a biocontrol agent, suppressing plant pathogens and promoting growth through the production of growth-promoting substances.
Fungi	*Trichoderma*	Protects against soilborne pathogens by outcompeting harmful fungi and degrading toxic compounds. Forms symbiotic relationships with plant roots, improving nutrient uptake, particularly phosphorus.

	spp. Mycorrhizal fungi (e.g., *Glomus* species)	
Actinomycetes	*Streptomyces spp.*	Produce antibiotics that suppress harmful pathogens and enhance soil health, promoting plant growth.
Yeasts	*Saccharomyces cerevisiae*	Used for fermentation processes in spices (e.g., in the production of fermented spice products).
Algae	*Chlorella*	Enhances soil fertility by fixing nitrogen and promoting the growth of spice plants.

Antioxidant Compounds in Spices

Spices are rich sources of antioxidants and hence have been used since ancient times both as medicine and flavoring agents. Besides fruits and vegetables, spices and herbs are great sources of antioxidants. The major bio-compounds found in spices are flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes and vitamins (Srinivasan *et al.* 2014). The antioxidant properties of spices are related to their chemical composition, primarily the presence of polyphenolic compounds. Many spices are highly potent antiseptics because they have antibacterial, antimicrobial, and even antiviral effects. A synergistic effect on oral and digestive bacteria was observed when cloves and asafoetida were used along with antibiotics.

Functions of Antioxidants

1. Antioxidants protect fats and oils in the food from oxidative degradation and prevent rancidity.
2. They retard the formation of toxic oxidation products and maintain the nutrient quality and shelf life of the food.
3. Antioxidants of spices are known to reduce the oxidative stress caused by high concentrations of free radicals in cells and tissues.
4. Antioxidants like malondialdehyde are effective in suppressing mutagenesis and carcinogenesis in the human body.

Table 2. Spices, their parts used, antioxidant composition and their mode of action

Spice	Parts Used	Antioxidant Compound	Mode of Action
Turmeric	Root (Rhizome)	Curcumin, Demethoxycurcumin	Curcumin neutralizes free radicals, reduces inflammation, and supports the immune system by modulating cellular pathways.
Ginger	Root (Rhizome)	Gingerol, Shogaol	Gingerol has antioxidant and anti-inflammatory effects, helps with digestion, and may improve blood circulation.
Cinnamon	Bark	Cinnamaldehyde, Coumarin	Cinnamaldehyde has anti-inflammatory, anti-diabetic, and antioxidant effects, and helps regulate blood sugar levels.
Cloves	Flower Bud	Eugenol, Beta-caryophyllene	Eugenol is a potent antioxidant and anti-inflammatory, supporting immune health and reducing oxidative stress.
Cardamom	Seed	Flavonoids, Terpenoids	Flavonoids and terpenoids have anti-inflammatory, antimicrobial, and antioxidant properties, aiding digestion.
Garlic	Bulb	Allicin, Diallylsulphide	Allicin provides antioxidant protection, helps with cholesterol management, and supports heart health.
Pepper	Fruit (Berry)	Piperine, Flavonoids	Piperine enhances nutrient absorption and has antioxidant, anti-inflammatory, and neuroprotective effects.
Saffron	Stigma (Flower)	Crocin, Safranal	Crocin and safranal are antioxidants that help reduce oxidative stress and may improve mood and brain function.

Mustard	Seed	Sinigrin, Allyl isothiocyanate	Sinigrin has antioxidant and anti-inflammatory effects, improving cardiovascular health and digestion.
Cumin	Seed	Cuminaldehyde, Thymol	Antioxidants in cumin support digestive health and provide anti-inflammatory benefits, enhancing metabolic function.

Use of Biofertilizers in Black Pepper (*Piper nigrum*) Cultivation

Black pepper *(Piper nigrum)* is a perennial climbing vine grown for its berries, which are used both as medicine and spice. Extensively grown in Kerala, Karnataka and parts of Tamil Nadu, making India the largest producer of the crop. The crop requires a humid tropical climate, requiring both rainfall and humidity.

From the study by Kandiannan *et al.* (2000), the production of black pepper increased post-adoption of integrated nutrient management (INM). To further improve the yield, the use of biofertilizer to fulfill one-third of the nutrient requirement of the plant was suggested by Thomas *et al*. (1991). Inoculation of the crop with vesicular-arbuscular mycorrhiza (VAM), N_2 fixing endophytes, Azospirillum, Azotobacter and Phospho-bacteria increases plant nutrient uptake, like nitrogen and phosphorus in an increase in growth, nutrient content of the crop and yield of the crop.

An experiment was conducted by the Indian Institute of Spices Research, Calicut (1996-97) on the use of three PGPMs, viz. Azospirillum, Phosphobacteria and vesicular-arbuscular mycorrhiza (VAM) in black pepper cultivation, where the plants responded well to the combined inoculation of PGPMs in terms of plant height, leaf area, biomass, dry matter and nutrient content. The growth of the vine and root formation was significant in the crop after applying biofertilizer (PGPMs). The crop produced more berries with the application of N2-fixing Azotobacter, Phosphobacteria and Azospirillum. Using biofertilizer increased shoot formation, biomass and dry matter of black pepper.

Table 3. Effect of biofertilizers on biomass, dry matter production and nutrient content of black pepper cuttings (18 months after treatment)

Treatment	Biomass (g/plant)	Dry matter (g/plant)	N%	P%	K%
Azospirillum	61	23	2.80	0.15	1.53
Phosphobacteria	73	24	2.87	0.16	1.57
VAM	74	24	2.98	0.16	1.63
Azo. + Phos.	89	27	3.10	0.17	1.66
Azo. + VAM	92	27	3.15	0.17	1.72
Phos. + VAM	132	31	3.17	0.18	1.80
Azo. + Phos. + VAM	146	34	3.27	0.21	1.94
Control	57	19	2.70	0.13	1.52
CD (0.05%)	22	5	0.17	0.05	0.30

Azo = Azospirillum; Phos = Phosphobacteria (Source: Kandiannan *et al.*, 2000)

Use of Biofertilizer in Fenugreek (*Trigonella foenum-graecum*) Cultivation

Sweet basil (*Ocimum basilicum*) is an annual, herbaceous spice crop used in various cuisines worldwide. It belongs to the Fabaceae family, characterized by green leaves, small white flowers, and pods containing brown seeds. Both leaves and seeds are used for various culinary and medicinal purposes. It reduces the risk of diabetes, high blood pressure, obesity, cancer and vascular inflammation. Produces secondary metabolites such as diosgenin, which is responsible for its medicinal effects.

From the study by Ghaleh *et al*. (2023), the role of micro-organisms like rhizobacteria and mycorrhizal fungi in improving growth, yield and diosgenin in fenugreek cultivation can be summarized as:

1. Vegetative Growth: The use of rhizobacteria and mycorrhizal fungi has resulted in increased root growth and penetration, which has increased the dry weight of roots. The leaf area also had a significant increase with an increase in the total chlorophyll content.
2. Diosgenin Content: There was an increase in the diosgenin content due to the action of *Sinorhizobium meliloti* and *Glomus versiforme.*
3. Mineral Content: With the use of rhizobium bacteria, maximum levels of nitrogen and phosphorus were achieved in shoots, whereas *Glomus versiforme* resulted in maximum levels of phosphorus and potassium.

It can be hence concluded that the use of the consortium of *Sinorhizobium meliloti, Azotobacter chroococcum and Glomus versiforme* as biofertilizers has resulted in increased vegetative growth and diosgenin production in fenugreek.

Use of Biofertilizers (PGPMs) in Turmeric (*Curcuma longa*) Cultivation

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial crop. It belongs to the Zingiberaceae family with large and oblong leaves and a bright yellow colored cylindrical rhizome, which consists of many medicinal properties. Turmeric is native to India and has uses in many areas. Turmeric has excellent anti-microbial, anti-inflammatory and anti-pyretic properties due to the presence of the secondary metabolite curcumin.

In the study done by Alshehri *et al.* (2024), on the role of rhizosphere bacteria on the growth, yield, and curcuminoid biosynthesis with the use of *Stenotrophomonas pavanii* has resulted in the following results:

1. Vegetative Growth: Application of *S. pavanii* has resulted in significant growth in the length of the stem, number of leaves, dry mass of leaves and stems and increased rooting was observed. Increase in rhizome number, increased diameter of the rhizome and increase in the dry mass of the rhizome were witnessed.

2. Photosynthetic Pigment Content: Controlled treatment of the crop with *S. pavanii* resulted in increased carotenoid and chlorophyll b content in turmeric, whereas there was a reduction in chlorophyll a content.

3. Nutrient Content: The use of biofertilizer has positively impacted the nitrogen and potassium content in the plant, whereas it has led to a drop in phosphorus content in the leaves of *C. longa*.

4. Curcuminoid Content: With the use of *S. pavanii,* there was a significant increase in the bioactive components in the rhizome of turmeric, viz., curcumin, demethoxycurcumin, and bidemethoxycurcumin. These bioactive compounds are responsible for the medicinal effects of turmeric.

Use of biofertilizer (PGPMs) in Rosemary (*Rosmarinus officinalis*) Cultivation

Rosemary (*Rosmarinus officinalis*) is a valuable spice, medicinal and aromatic herb used worldwide. Rosemary has a square stem with needle-like leaves and is a source of fragrant essential oil. Rosemary originated in the Mediterranean region, belonging to the family Lamiaceae. It is rich in antioxidants and rosemary oil has many medicinal effects, such as anti-bacterial, anti-fungal, anti-cancerous properties and stimulates blood circulation.

In a study done by Shehata *et al.* (2019), plant growth-promoting microbes like Azotobacter, Azospirillum and Klebsiella have been used as biofertilizer, bacteria *Bacillus megatherium* have been used to stimulate plant growth-promoting hormones like IAA, cytokinin and gibberellins and Arbuscular Mycorrhizal Fungi (AMF) help in nitrogen fixation. The following were the observations recorded from the yield made from the use of biofertilizer in rosemary cultivation:

1. Vegetative Growth: A combination of Arbuscular Mycorrhizal Fungi and *Bacillus megatherium* has resulted in a significant increase in growth of plant height, an increase in dry weight and fresh weight.
2. Chemical Constituents: A combination of Arbuscular Mycorrhizal Fungi (AMF), *Bacillus megatherium* and *Azospirillum brasilense* has resulted in the highest values of chemical constituents.

3. Volatile Oil: The triple mixture of Arbuscular Mycorrhizal Fungi (AMF), *Bacillus megatherium* and *Azospirillum brasilense* has led to the production of the highest oil yield percentage per plant.

Further Suggestions

The use of microbes in spice cultivation started in the last two decades. Much research has been done on this topic, and the findings have concluded that the use of PGPMs as biofertilizers has enhanced the yield of the crop and the nutrient content in it. Hence, the use of biofertilizer for the production of spices should be promoted as it improves the quality of yield, leading to higher market prices and environmental sustainability.

Conclusion

The economic importance of spices in the world and their growing demand due to the growing population require extensive cultivation. These crops are highly affected by biotic and abiotic factors. Microbes play a crucial role in improving plant health and suppressing pathogenic attacks on them. This will open up opportunities to manage the spice plant disease and improve the quality and quantity of the yield and a step towards sustainable agriculture. Therefore, the positive impact of microbes in spice cultivation has caught the interest of researchers to further improve the genetic base of microbes and crops.

In conclusion, spices have high importance in the national economy because of their versatility in use, from flavor enhancers in food to components in medicines. India is the largest producer and consumer market for spices. Hence, the production should be done in the most sustainable way using biofertilizers that are locally available and suitable for soil health, which creates a beneficial ecosystem between the farmer, crop, microbe and soil.

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