Original Research Article

Enhancement of Beneficial Soil Microflora and Disease Suppression by Chitosan Application in Turmeric

ABSTRACT

The present study assessed the population of nitrogen fixers and phosphate solubilizers in the rhizosphere of turmeric plants, and suppression of leaf blotch disease in turmeric caused by *Taphrina maculans*, a soil borne pathogen, in response to chitosan application.The treatments includedfoliar spray of chitosan 2gL^{-1}at monthly intervals (F_1), foliar spray of chitosan 4gL^{-1}at bimonthly intervals (F_2), soil drenching of chitosan 2gL^{-1}at monthlyinterval (S_1) and soil drenching of chitosan 4gL^{-1}at bimonthly interval (S_2). The untreated plants served as the control. The population of beneficial soil microbes were assessed and soil application of chitosan was observed to have better effect over foliar application with respect to the population of nitrogen fixing bacteria. Morepopulation of nitrogen fixing bacteria was recorded the in the soil where chitosan 4gL^{-1} was applied as soil drenching at bimonthly interval (S_2). The population of phosphate solubilizers were significantly higher over the control in all the chitosan treatments irrespective of the mode of application. However, the higherpopulation of phosphate solubilizers were observed, when chitosan 2 g $L^{-1}$was given as foliar spray at monthly intervals (F_1).The chitosan treated plants were also observed to have significantly lower incidence of leaf blotch disease compared to control plants. The use of chitosan as a plant biostimulant protects the plant from soil borne pathogens and increases the population of beneficial soil microflora, which have a role inimproving the nutrient uptake by plants thereby enhancing the plant growth and yield.

Keywords: Turmeric, Chitosan, Nitrogen fixing bacteria, Phosphate solubilizers, Leaf blotch

1. INTRODUCTION

Soil amendments, either organic or inorganic when added to the soil improve its physiochemical and biological properties. It provides a favourable environment for plant growth by improving the soil structure, soil microflora, water holding capacity and availability of nutrients. Organic amendments are a rich source of organic matter and nutrients. It facilitates the growth and activity of soil microorganisms. Increased activity of soil microbes promotes efficient nutrient cycling and suppress soil-borne pathogens. Soil microbes decompose organicmatter, during whichthey produce enzymes and metabolites that are capable of breaking down salts and other harmful compounds, thus improving soil health [1].

According to Deng et al.[2], organic amendments could increase rhizosphere microbial diversity by providing additional nutrient sources. Organic amendments could facilitatebeneficial symbiotic relationship between plants and soil microflora, such as mycorrhizal fungi and nitrogen-fixing bacteria. Alori *et al*. [3] observed that organic amendments like compost, poultry manure and green manure from *Tithonia diversifolia*improved number and diversity of fungi and bacteria. The plant growth seems to be the outcome of better soil physical properties *viz.,*pore size distribution, particle size, porosity, water-holding capacity and enhanced populations of soil microbes (fungi, bacteria, actinomycetes).

Chitosan is a plant biostimulant that evokes plant growth promotion, soil conditioning, metabolite elicitation and defense responses in plants. Chitosan, extracted from exoskeletons of crustaceans, certain insects and fungal cell wall is a natural, safe and cost effective biopolymer obtained from the deacetylation of chitin, a long chain polymer of N-acetyl-glucosamine [4]. Chitosan could be applied to plants as seed priming, soildrench or asfoliar spray. Chitosan when applied to soil has shown great effect on plant growth due to its ability to improve of soil fertility and enhancement of mineral nutrients absorption by plant [5].Due to the presence of nitrogen (~8.9%–9.5%) in its amino group, chitosan serves as a source of nitrogen to the plants. Also, when applied to the plant it

increases the mineral nutrients uptake of nitrogen, phosphorous, potassium, calcium and magnesium[6].

Sharp [7] reported that the addition of chitosan alters rhizosphere conditions to shift the microbial balance in favour of beneficial organisms and detrimental to plant pathogens. The primary function of these beneficial bacteria is to decompose organic matter, provide nutrients to crops, improve soil structure etc. Healthy populations of beneficial bacteria can help suppress pathogens and promote plant growth and increase the yield[8].In a study conducted by Dzung *et al*. [9], it was found that chitosan spray resulted in improvednutrient uptake.The synergetic effects of many factors, such as suppression of plant diseases, insects, and nematodes, increased biomass and activities of beneficial microbes, improved physical structure of soil and nutrient availability and direct plant growth stimulation makes chitosan as a good soil amendment [10].

Chitosan has been reported to have a positive effect on rhizobacterial growth, where itpossesses a symbiotic relation with growth promoting rhizobacteria, thus triggered germination rate and improving plant nutrient uptake [11].Nitrogen fixers and phosphate solublizers are important groups of plant growth promoting rhizobacteria having unique role in agriculture. *Azotobacter chroococcum* and *Azospirillumbrasilence*, are two free-living aerobic nitrogen fixing bacteria that can be found in most soil and have the ability to convert inert N_2 into available forms for plants. *Bacillus megaterium* and *Pseudomonas fluorescens* are notable for the ability to solubilize unavailable phosphates in soil, as well as produce a wide variety of metabolites like auxin[12].

The present study was undertaken to assess the population of beneficial soil microflora(nitrogen fixing and phosphate solubilising bacteria) in the rhizosphere of turmeric plants in response to chitosan, when applied both as soil amendment and foliar spray.

2. MATERIALS AND METHODS

Turmeric plants were grown in farmer's field in Trivandrum district, Kerala during June 2021 to January 2022. The varieties, Sobha and Sona released from Kerala Agricultural University were used for the study. The 45 day old protray plantlets were transplanted to the main field at a spacing of 25cm x 25cm in plots of size 3m x 1m and the field was laid out in Randomized block design with four replication. Crop was raised organically as per Package of Practices recommendations (Organic) [13]. Chitosan treatments were given till 5 months after transplanting (MAT). The treatments includedfoliar spray of chitosan $2gL^{-1}$ at monthly intervals (F_1), foliar spray of chitosan $4gL^{-1}$ at bimonthly intervals (F_2), soil drenching of chitosan $2gL^{-1}$ at monthly interval (S_1) and soil drenching of chitosan $4gL^{-1}$ at bimonthly interval (S_2). The plants that were not exposed to either mode of application of chitosan served as the control. Plants were harvested at 7 months after transplanting.Soil samples were collected from the rhizosphere of turmeric plants in sterile polythene bagsat the time of harvest. The population assessment of free living nitrogen fixing microorganisms and phosphate solubilizers of rhizosphere soil, collected from the field was carried out by serial dilution followed by plating [14]. Nitrogen fixing bacteria from the soil samples were counted after growing in different N-free agar media such as N-free malate bromothymol blue (NFB) and Jensen's media. The isolates of phosphate solubilizing bacteria were counted onPikovskaya'sagar.The population of beneficial soil microflora was expressed in log cfu g^{-1} soil from three replicates.Crop was monitored throughout the growth period and incidence of leaf blotch disease, and disease intensity was assessed by using 0 to 6 disease rating scale [15]. The data were subjected to analysis of variance using package KAU GRAPES [16].

3. RESULT AND DISCUSSION

3.1 Effect of chitosan on beneficial soil microflora

The nitrogen fixing bacteria could be seen as sugar like crystals on the media. Significant difference was noticed in the population of nitrogen fixing bacteria among the treatments (Table 1). The growth of nitrogen fixing bacteria showed similar trend in both the media. In the variety Sobha, nitrogen fixing bacteria was observed to be the highest in S_2 (soil drenching with chitosan 4g L^{-1}at bimonthly interval) in both NFB (5.02 log cfu g^{-1}) and Jensen's media (4.89 log cfu g^{-1}). In the variety Sona also same treatment recorded the highest number of colony forming units in NFB (5.12 log cfu g^{-1}) and Jensen's media (5.12 log cfu g^{-1}). The lowest population of nitrogen fixing bacteria was observed in control treatment in both the varieties.

Table 1. Population of nitrogen fixing bacteria in response to chitosan application

Treatments	Population of nitrogen fixing bacteria (log cfu g^{-1})			
	NFB medium		Jensen's medium	
	Sobha	Sona	Sobha	Sona
F_1: 2gL^{-1} monthly foliar application	4.24 ± 0.06^{c}	4.32 ± 0.13^{c}	4.41 ± 0.19^{b}	4.45 ± 0.17^{b}
F_2: 4gL^{-1} bimonthly foliar application	4.07 ± 0.11^{d}	4.21 ± 0.24^{c}	4.36 ± 0.16^{b}	4.40 ± 0.13^{b}
S_1: 2gL^{-1} monthly soil application	4.63 ± 0.06^{b}	4.67 ± 0.10^{b}	4.19 ± 0.17^{b}	4.56 ± 0.28^{b}
S_2: 4gL^{-1} bimonthly soil application	5.02 ± 0.05^{a}	5.12 ± 0.07^{a}	4.89 ± 0.06^{a}	5.12 ± 0.10^{a}
Control (with no chitosan treatment)	4.00 ± 0.07^{d}	4.19 ± 0.10^{c}	4.14 ± 0.16^{b}	4.28 ± 0.16^{b}
SEm (±)	0.043	0.081	0.09	0.103
CD (0.05)	0.135	0.254	0.285	0.323
CV	1.69	3.10	3.564	3.891

The values are mean of **3**replications. Values that are followed by the same letter in a column do not differ significantly between them

Soil application of chitosan was observed to have better effect over foliar application with respect to the population of nitrogen fixing bacteria. When the same concentration of chitosan was applied as foliar spray and soil drenching, the later was found to be more effective in improving the microbial population. The multiplication and activities of soil bacterial population would have more when chitosan was directly applied to the soil rather than when applied on the leaves. Chitosan when applied tosoil could alter the soil properties in favour of microbial growth and multiplication, in consensus with the findings of Xu and Mou [10], who reported that chitosan could provide a carbon source for microbes in the soil.

Nitrogen is the most essential and restrictive nutrient for plant growth. The unavailable atmospheric nitrogen converts to plant available form as ammonia through nitrogen fixation by a group of nitrogen fixing bacteria in the soil. Nitrogen fixing is essential for plant nutrition by increasing N uptake by the plants and playing a significant role as plant growth promotion [17]. The improvement in the count of nitrogen fixing bacteria by chitosan application in our study indirectly would indicate its effect on plant growth promotion.

The population of phosphate solublizers also showed significant difference among the treatments tried (Table 2). In Sobha, the highest number of colony forming units of phosphate solubilizing bacteria (3.32 log cfu g^{-1}) were recorded in F_1 and this was on par with all other treatments except control. In Sona, the highest value (3.42 log cfu g^{-1}) was recorded in F_1 and on par with F_2 and S_2. The control treatment recorded a significantly lower value for phosphate solubilizing bacteria in both the varieties.

Table 2. Population of phosphate solubilizing bacteria in response to chitosan application

Treatments	Population of phosphate solubilizing bacteria (log cfu g^{-1})	
	Sobha	Sona
F_1: 2gL^{-1} monthly foliar application	3.32 ± 0.28^{a}	3.42 ± 0.10^{a}
F_2: 4gL^{-1} bimonthly foliar application	3.25 ± 0.24^{a}	3.36 ± 0.10^{a}
S_1: 2gL^{-1} monthly soil application	3.00 ± 0.00^{a}	3.10 ± 0.17^{b}

S_2: 4gL^{-1} bimonthly soil application	3.10 ± 0.17^{a}	3.30 ± 0.00^{ab}
Control (with no chitosan treatment)	2.00 ± 0.00^{b}	2.10 ± 0.17^{c}
SEm (±)	0.105	0.074
CD (0.05)	0.33	0.232
CV	6.175	4.167

The values are mean of 3 replications that are followed by the same letter do not differ significantly between them

*Burkholderia gladioli,*a phosphate-solubilizing bacteria considerably improved its phosphate-solubilizing capacity when cultured in fermented chitosan medium, which further increased the amount of available phosphorus when amended in the soil[18].Amerany, *et al*[19] in his study chitosan 1 mg per plant was applied to the soil in the transplant cavity after three weeks of transplanting of arbuscular mycorrhizal fungi (AMF) inoculated tomato (*Solanum lycopersicum*) seedlings and AMF colonization frequency and intensity reached to higher values after 12 weeks of growth.

Phosphorus is the second most essential nutrient element for plants growth. Phosphorus plays an important role in photosynthesis, development of good root system, respiration, energy storage and transfer etc. The improved count of phosphate solublizers by chitosan application helps in enhanced phosphorus uptake by plants and thus leads to better plant development.In the present study the population of phosphate solublizers were found to be significantly higher over the control in all the chitosan treatments irrespective of foliar and soil application in both the varieties.

Kerala Agriculture University recommends the application of *Azospirillum* and phosphate solubilizing bacteria (20 g/ bed of 3m^2) as nutrient supplement in turmeric along with chemical fertilizers [20].From the findings of our study it can be said that chitosan can be used as an alternative to meet this recommendation. In our experiment we found that chitosan application enhanced the count of nitrogen fixing and phosphate solubilizing bacteria in the root zone. These two beneficial soil microbes are actively involved in facilitating plant's nutrient uptake. Thus the use of chitosan as plant biostimulant can supply the nutrients to the plants as well as improve the population of beneficial microflora in soil.

3.2 Effect of chitosan on disease incidence

The incidence of leaf blotch disease caused by *Taphrina maculans* was noticed in the field at 6 MAT and significant variation was observed with respect to per cent disease indexamong the treatments in both the varieties (Table 3). Significantly lower disease index was recorded in F_1 (12.78 %) in Sobha and F_2 (14.44 %) in Sona and found to be on par with all other chitosan treatments. The highest per cent disease index was noticed in control plot in both the varieties. Leaf blotch disease caused by *Taphrina maculans* is a serious disease of the turmeric leaves causing a significant decrease in yield due to loss of photosynthetic properties. The disease is soil and seed borne and the pathogen survive in soil on infected plant debris.

Table 3. Per cent incidence of leaf blotch disease in response to chitosan application

Treatments	**Per cent disease index**	
	Sobha	**Sona**
F_1: 2gL^{-1} monthly foliar application	12.78 ± 2.84^{b}	15.00 ± 1.36^{b}
F_2: 4gL^{-1} bimonthly foliar application	15.56 ± 3.42^{b}	14.44 ± 1.57^{b}
S_1: 2gL^{-1} monthly soil application	16.11 ± 2.08^{b}	15.55 ± 3.14^{b}

S_2: 4gL^{-1} bimonthly soil application	13.33 ± 1.36^{b}	17.78 ± 2.08^{b}
Control (with no chitosan treatment)	46.11 ± 2.08^{a}	48.34 ± 1.36^{a}
SEm (±)	1.091	1.125
CD (0.05)	3.361	3.468
CV	10.5	10.129

The values are mean of 4 replications that are followed by the same letter do not differ significantly between them

Anusuya and Sathiyabama[21]reported that turmeric plant treated with chitosan (0.1% w/v spraying on leaves) showed increased resistance towards rhizome rot disease caused by *Pythium aphanidermatum*.In a study conducted to determine the effects of chitosan on *Colletotrichum* anthracnose on papaya fruit,more than 60% control of anthracnose was reported when chitosan is applied before pathogen inoculation [22]. Liu et al.[23] demonstrated that chitosan protects rice seedling from sheath blight caused by *Rhizoctonia. solani*and causes a 66-91% inhibition of lesion length.

Chitosan was also found to have effect on disease suppression by the way of enhancing systemic resistance of the plant. The mechanism of action of chitosan is through direct toxicity or chelation of nutrients and minerals from the pathogen. The biopolymer properties of this compound also helps to form physical barriers around the penetration sites of pathogens thus, preventing them from spreading to healthy tissues. Chitosan induces local and systemic reactions that involve in signaling cascades and activation of defenses-related antimicrobial compounds and proteins [24].

In the present study, the application of chitosan was found to be effective in reducing the incidence of leaf blotch disease. The foliar spray and soil application of chitosan may help to reduce the population of harmful pathogens in the soil and thus protects the plant from disease incidence.

4. CONCLUSION

Chitosan, is a natural, safe and cost effective biopolymer extracted from exoskeletons of crustaceans, certain insects and fungal cell wall. The addition of chitosan alters rhizosphere conditions to shift the microbial balance in favour of beneficial organisms and detrimental to plant pathogens. In the present study, the use of chitosan as foliar spray or soil drenching, increased the population of beneficial soil microflora *viz.* nitrogen fixing bacteria and phosphate solublizers. These microbes play a major role in plant's nutrient uptake andthat may reflects on plant growth and yield. Chitosan application was also found to be effective in controlling the leaf blotch disease caused by *Taphrina maculans*.

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