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SUBSTRATE DYNAMICS: DEVELOPMENTS AND ISSUES

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ABSTRACT

Rhizosphere modification through root exudation is an important attribute that regulates not only the availability of nutrients in soil, but also their acquisition by plants, besides soil microbial composition. Limited studies on multi-inoculation loaded substrate dynamics with respect to rhizosphere physico-chemical and biological transformations have displayed some promising results in variety of crops. But, long term studies on sustainable impact of substrate on production as well as quality are still in the pipeline. A major breakthrough of late has emerged in this context , is the rhizosphere specific microbial consortium having multiple microbes capable of performing diverse functions. Such microbial consortium needs to be tailored as per crop specific demand so that substrate dynamics becomes more crop responsive with an additional lasting effect on soil biological health.

INTRODUCTION

External agricultural inputs such as mineral fertilizers, organic amendments, microbial inoculants, and pesticides are applied with the ultimate goal of maximizing productivity and economic returns, while side effects on soil organisms are often neglected (Katyal 2003; Kumar and Singh 2009; Gangata and Kaushal 2009; Shivran and Jat 2013). Mineral fertilizers have limited direct effects, but their application can enhance soil biological activity via increases in system productivity, crop residue return, and soil organic matter (Hartz *et al.* 2000; Sankar *et al.* 2009; Khan *et al*. 2009 ; Khandelwal *et al*. 2013). Another important indirect effect especially of nitrogen fertilization is soil acidification, with considerable negative effects on soil organisms (Chhonkar 2003). The outcome of a long-term fertilizer experiment in rice established that a balanced application of nutrients promoted microbial biomass through improved diversity of the microbial community (Zhang and Wang 2005).

Inoculant application research, on the other hand, is increasing with a focus on co-inoculation with several strains or mixed cultures enabling combined niche exploitation, cross feeding, complementary effects, and enhancement of one organism's colonization ability when co-inoculated with a rhizosphere competent strain (Goddard *et al*. 2001; Upadhyay and Kushal 2008; Singh *et al.* 2008). The outcome of studies like population diversity analysis of fluorescent *Pseudomonas* within the plant's rhizosphere, which helped to discriminate flax (*Linum ustitatissinum* L.) and tomato (*Lycoperscion esculentum* Mill.) isolates (Lemanceau *et al*. 1995), could be befittingly exploited to synthesize a rhizo-competent dynamic substrate suiting diverse requirements of a specific crop. Later studies (Johnston 2009; Siasou *et al.* 2009) established that AM inoculation in wheat increased the biocontrol efficiency of *Pseudomonas fluorescence* on account of increased synthesis of 2,4-diacetylphloroglucinol by the latter in the presence of soluble C in the soil. In another study, Rengel *et al*. (1996) observed that the total number of bacterial colony forming units increased in the rhizosphere of Zn-efficient genotypes of wheat under Zn-deficiency and in Mn-efficient genotypes under conditions of Mn-deficiency. In contrast, a Zn-deficiency treatment acted synergistically with the number of fluorescent *Pseudomonas* in the rhizosphere. Substrates are nothing but the carrier of microbial inoculants. Organic amendments such as manure, compost biosolids, and humic substances provide a direct source of C for soil organisms as well as an indirect source via increased plant growth and plant residue return (Bunemann *et al*. 2006). Organic and integrated production systems offer alternatives to conventional production systems (Curl *et al*. 2002; Peck *et al*. 2005). However, integrated production methods are yet to attain the same widespread farmers' acceptance as organic production methods.

Substrate dynamics - Developments

Consistent efforts are being made to find alternatives to conventional fertilizers, media and practices, although chemical properties of formulated substrates may affect plant growth and nutritional response in varied ways viz., i. improvement in soil hydraulic properties, ii. maintenance of better available pool of nutrients, and iii. establishment of dynamic soil microbial environment, more suited to

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crop requirement (Dutt *et al.* 2002; Altland and Buamscha 2008). The origin of a substrate and its pH are considered two most important guiding principles in developing a substrate dynamic to plant's rhizosphere in addition to physical stability, ease in rewetting ability to withstand compression, and low shrinkage rate over time (Roose and Haase 2000; Altland 2006). Dutt and Sonawane (2006) observed excellent performance of chrysanthemum (*Chrysanthemum indicum* L.) on a substrate containing cocoa-peat-compost-rice husk. Recently, studies (Buamscha *et al*. 2007; Altland *et al*. 2008) documented that DFB (Douglas Fir Bark) alone provided sufficient micronutrients for annual vinca (*Catharanthus roseus* L.) grown at low pH (4.6-5.5). While Hernandez-Apaolaza *et al.* (2005) suggested that the use of pink bark in coconut (*Cocos nucifera* L.) coir-based media formulations served as one alternative of recycling waste materials. Fisher *et al*. (2006) suggested peat-based substrate treated with lime to adjust pH within an optimum range.

Coir dusts with a particle size distribution similar to peat showed comparatively higher aeration and lower capacity to hold total and easily available water. An air-water balance similar to that in peat became apparent in coir dust at a comparatively lower coarseness index (29% vs. 63% by weight in peat). Stepwise multiple regression analysis showed that particles with diameters in the range of 0.125 to 1 mm had a remarkable and highly significant impact on the physical properties studies, while particles < 0.125 mm and > 1 mm had only a slight or non-significant effect (Abad *et al.* 2005). Four types of media [coir, 1 coir: 2 peat (by volume), peat, and sandy loam soil] were evaluated by Merhaut and Newman (2005) for their effects on plant growth and nitrate (NO_3^-) leaching in the production of oriental lilies (*Lillium* L.) 'Starfighter' and 'Casa Blanca'. Results indicated that the use of coir and peat did not significantly influence plant growth (shoot dry weight) relative to the use of sandy loam soil. However, substrate type influenced the amount of NO_3^- leached through the media and N accumulation in the shoots for 'Starfighter', but not for 'Casa Blanca'.

Various recipes for potting mixture exist that do not contain synthetic components (Kuepper and Adam 2003; Salifu *et al*. 2006). Koller *et al*. (2004) used several plant- and animal-based substrates in the production of vegetable transplants. They stipulated that plant-based substrates should be mixed into the potting medium 2 weeks before sowing seed to prevent damage. Worm castings of EF have been tested as a component of media for organic production to tomato, and it was found that seedling development improved as percent of worm castings in the medium increased (Ozores-Hampton and Vavrina 2002). Regardless of their origin these materials and practices are generally referred to as being alternatives to conventional fertilizers, media, and practices. To be accepted as commonplace in the industry, alternative materials and practices must be compared to existing conventional materials and practices (Russo 2005). For example, a typical substrate tested in azalea (*Rhododendron atlanticum*) and camellia (*Camellia japonica*) (Merhaut *et al*. 2006; Blythe *et al*. 2006) consisted of 5 sphagnum peatmoss; 4 pine bark (6.7-9.5 mm): 1 washed builders sand (by volume); amended with dolomite 65 at a rate of 0.59 kg m^{-3} and ultrafine calcium sulfate at a rate of 0.59 kg m^{-3}, mixing the substrate and amendment.

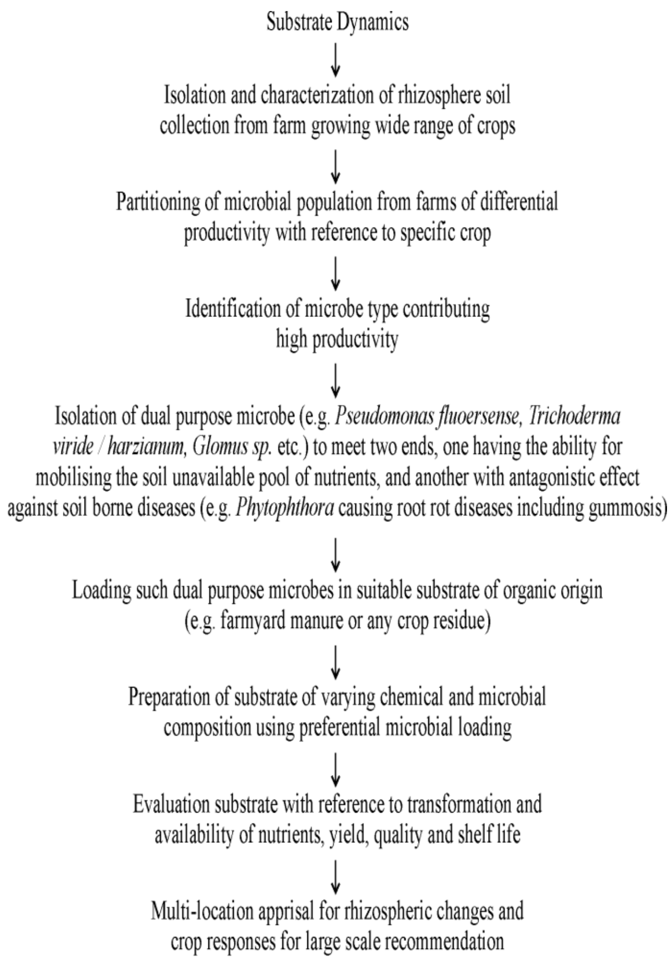

Fig.1**.** Schematic representation of steps involved in development of microbially loaded substrate

The nutrient composition (mg L^{-1}) was observed as: 1306 Ca, 019 Mg, 2.62 Fe, 0.59 Mn, 0.75 Zn, 0.11 Cu and 0.01 Mo. The substrate was later mixed with different controlled release fertilizers, CRF viz., Omocote (24-4-9), Nutricote (18-6-8), Multicote (17-5-11 + minor nutrients), and Polyon (17-5-11 + micro nutrients), all having 365 days release formulations in terms of highly acidic pH and particle size distribution.

Crop residue is another option to be used as a strong support to substrate in any substrate dynamics studies. For example, in India, crop residues available are estimated to be 600 million Mg. Rice and wheat are two major crops, generating around 250 million Mg of residues (Selvakumar *et al.* 2008). Decomposing paddy straw is a problem because it contains approximately 40% cellulose, 20% hemicellulose and 12% lignin, and has high C: N. Several strains of mesophilic and thermophilic microorganisms were screened for utilization of paddy-straw. Four fungi, *Phanerochaete sporium*, *T. viride*, *Aspergillus nidulus*, and *A. awamori* were identified by Selvakumar *et al.* (2008) to carry out solid state fermentation of paddy straw; all combinations were good. The process involved construction of perforated brick tanks for proper aeration for composting of paddy-straw. The straw was supplemented with poultry droppings (8: 1) or urea at 0.5% to bring down the C: N ratio of the straw. A tank of 1 m^3 can accommodated 80 kg of straw. Rock phosphate (1%) along with inoculum containing 4 fungi was applied at 0.5 kg ton^{-1} straw and mixed in the tank. Moistened paddy with sufficient water, and within 2-3 months, compost with a C: N ratio of 15: 1 can be successfully obtained. Such an attempt needs to be replicated using other crop residues as substrate. Siddiqui *et al*. (2008) observed that the application of *T. harzianum*-inoculated rice straw compost not only improved the morpho-physiological characters of okra but reduced the wet rot incidence compared to control, and offered an environmentally friendly alternative to inorganic fertilizers/fungicides, resulting in higher yield.

Coinoculation or combined inoculation of different microbe types is another area which can be gainfully exploited in formulating the microbially-rich substrate, provided that information on the synergism between different microbes is known (Marschner *et al*. 2004). In the past, a number of studies have suggested the coinoculation of different microbes, which can be summarized as: *A. brasilense – P. striata/B. polymyxa* in sorghum (Alagawadi and Gaur 1992), *A. lipoferem – Agrobacterium radiobacter*/*A. lipoferem-Arthrobacter mysorens* in barley (Belimov *et al*. 1995), *A. brasilense – Rhizobium* in lentil (Yadav *et al.* 1992) and chickpea (Fabbrie and Del Gallo 1995), *A. brasilense – A. chroococcum – Klebsiella pneumoniae – R. meliloti* in alfalfa (Hassouna *et al.* 1994), *A. brasilense – R. leguminosarum* in soybean (Neyra *et al*. 1995), and *A. brasilense*/*Streptomyces mutabilis – A. chroococcum* in wheat (Elshanshoury 1995). Many studies on coinoculation of microbes involving AM fungi and bacteria have also been suggested for improvement in both yield and quality. These include: *A. brasilense – G. fasciculatum* in wheat (Gori and Favilli 1995), strawberry (Bellone and de Bellone 1995); *A. brasilense – Pantoea dispersa* in sweetpepper (Amor *et al*. 2008); and *A. chroococcum – G. mosseae* in pomegranate (Aseri *et al*. 2008).

Various steps involved in developing a substrate could be summarized through a flow diagram (Fig. 1) in fulfilling rhizosphere's diverse requirements.

Microbial Consortium – a step forward

Growth promoting microbes were isolated from rhizosphere (0-20 cm) for development of MC through extensive soil sampling (from the rhizosphere of as many as 110 plants) at the experimental site. The microbial diversity existing within rhizosphere soil was isolated following standard procedures, and characterized the promising microbes for their nutrient mobilizing capacity through laboratory-based incubation study using the same experimental soil. The efficient microbes viz.,*Azotobacter chroococcum*(asymbiotic N-form), *Bacillus mycoides*(K-solubilizer), *Pseudomonas fluorescens* (P-solubilizer), *Bacillus polymyxa* (P-solubilizer), and *Trichoderma harzianum*(P-solubilizer) were finally identified. Pure culture of these microbes in value added form was developed in broth, and prepared a mixture called MC. The compatibility amongst these microbes was tested by thoroughly their population dynamics in consortium mode which showed no antagonism amongst them upto 90 days of laboratory oriented incubation study (Srivastava *et al.* 2012).

The developed microbial consortium was evaluated in nursery plants, both on seedlings for 45 days and in buddlings for 124 days, using a total of 354 plants. Out of these 354 plants, 172 plants in 13 replications (each with 4 units) were treated and other 172 plants in 13 replications were kept as untreated control (Table 1). The response of microbial consortium on rough lemon seedlings showed a significant increase in various growth parameters

(9.59 g root weight, 24.86 g shoot weight, and 11.9 mm stem diameter on per plant basis) over control (2.99 g root weight, 9.08 g shoot weight, and 8.6 mm stem diameter on per plant basis). Similar observations were made on buddlings also (Wu and Srivastava 2012). The inoculation with microbial consortium brought a significant change in available supply of different nutrients in soil and microbial biomass nutrients (Table 2). A significantly higher soil fertility status with microbial consortium treated plants (123.4 N – 16.2 P – 13.7 Fe – 10.2 Mn – 0.88 Zn mg kg^{-1}) was observed compared to untreated control (116.2 N – 13.2 P – 8.8 Fe – 6.7 Mn – 0.62 Zn mg kg^{-1}). Similarly, microbial biomass nutrients were higher in the rhizosphere treated with microbial consortium (147.7 mg kg^{-1} C_{mic}, 34.1 mg kg^{-1} N_{mic} and 17.8 mg kg^{-1} P_{mic}) than untreated control (119.8 mg kg^{-1}, C_{mic}, 21.8 mg kg^{-1} N_{mic} and 13.5 mg kg^{-1} P_{mic}). The above observations strongly supported the effectiveness of microbial consortium in improving chemical and biological indices of citrus rhizosphere.

Table 1: Response of microbial consortium in nursery plants (Pooled data of 2 seasons)

Treatments	Root weight (g)	Shoot weight (g)	Root:shoot	Stem diameter (mm)
Seedlings (Period : 45 days)				
Control	2.99	9.08	1.1.037	8.61
Treated	9.59	24.86	1:2.85	11.9
LSD (*P = 0.05*)	3.65	5.63	-	1.43
Treated	11.76	26.41	1:2.20	28.51
LSD (*P = 0.05*)	2.03	5.635		2.02

Source : Wu and Srivastava (2012)

The treatment combination of ¾P + AM + N was observed the best treatment with reference to better growth and yield of high quality fruits of ‘Mosambi’ sweet orange suggesting the compatibility of biofertilizers (AZO) and AM inoculation in combination with chemical fertilizers for better growth, yield and fruit quality. Such observations in the long term are expected to cut down the cost of chemical fertilizers, particularly N and P and building up fertility by maintaining better soil physical conditions. High efficiency of *Azospirillum* for fixing nitrogen and better mobilisation of fixed phosphorus by AM even at high temperatures can make these highly suited for Mosambi sweet orange (Manjunath *et al*. 1983).

Table 2: Changes in soil fertility indices in response to inoculation with microbial consortium (Pooled data of 2 seasons)

	Soil fertility (mg kg^{-1})						
	N	**P**	**K**	**Fe**	**Mn**	**Cu**	**Zn**
Control	116.2	13.2	166.7	8.8	6.7	1.12	0.62
Treated	123.4	16.2	169.7	13.7	10.2	1.16	0.88
LSD (*P = 0.05*)	3.95	2.0	NS	1.75	1.35	NS	0.12
	Microbial biomass nutrients (mg kg^{-1})						
	C_{mic}		N_{mic}		P_{mic}		
Control	119.8		21.8		13.5		
Treated	147.7		34.1		17.8		
LSD (*P = 0.05*)	9.85		2.5		1.25		

- Computed on the basis of analysis after 162 days of inoculation

C_{mic}, N_{mic}, and P_{mic} stand for microbial biomass-C, microbial biomass-N and microbial biomass-P, respectively.

Source : Wu and Srivastava (2012)

However, these developments on the subject are still in developing stage. Many issues still continue to hit the researchers in way of an effective use of substrate dynamics concept during the pre-evaluation of various crops.

MAJOR ISSUES

Despite many cutting edge technologies addressing a variety of core issues of nutrient biochemical response in relation to varying nutrient supply systems (through INM modules) especially under agropedological conditions facing multi-nutrient deficiencies and establishing the causal relationship between the physico-chemical and microbiological changes within rhizosphere and to be able to coordinate changes in shoot system (changes in canopy size and fruit yield i.e. yield efficiency), are very much imperative that are seemingly most sensitive to various combinations of remediative treatment. Nutrient dynamics is another virgin area where limited attempts have been made using citrus as test crop. Amongst different nutrients, Zn has attracted worldwide investigation from various angles. The changes in rhizosphere bring different simultaneous changes in microbial diversity *vis-à-vis* C_{mic}, N_{mic}, P_{mic} and nutrient regime especially for diffusion limited nutrients like P, Zn, Fe, Mn, etc. has to find serious considerations in any nutrient management program that involves INM-based corrective treatments. Additionally, the conditions under which crops are most likely to respond to corrective Zn-treatments are still not fully understood. The role of Zn in perennial crops with respect to flowering, fruit set, fruit quality (external and internal) and juice shelf life; models defining the critical periods of Zn-supply to assure sustained response and its uptake for helping the management decision under different citrus-based cropping systems; and devising means for improved Zn-uptake efficiency need to be attempted to unravel many of management, many more issues are yet to be attempted with respect to INM-based crop production vis-à-vis rhizosphere dynamics. Studies on the complexities involved with Zn-nutrition under INM-based production management.

Out of different soil properties, the microbial biomass is the one biological property of soil that undergoes immediate change in response to fertilizer like input. Studies, therefore, need to be undertaken with a view to explore the possibility whether microbial properties could be used as a potential tool for finding out soil fertility constraint instead of available supply of nutrients in soil. Simultaneously, eye should be kept on long term changes in total carbon pool of soil to arrive at the logistic conclusion that sequestration of carbon through improved production level could rejuvenate the lost productivity potential of nutritionally depleted soil. However, it remains to be further established that any change in microbial diversity within the rhizosphere is brought about with different sources of substrate, and if there is any, how the nutrient dynamics is associated with orchard productivity. With the availability of more technical know-how on efficient use of bulky organic manures, prolonged shelf life of microbial bio-fertilizers, and better understanding on crop-mycorrhiza symbiosis with regard to nutrient acquisition and regulating the water relations, a more effective integrated crop production system could be evolved in future. The molecular approach to breeding of mineral deficiency resistance and mineral efficiency would facilitate to produce nutritionally efficient biotypes in order to maximise the quality production on sustained basis.

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