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Bioprotectant with multifunctional microorganisms: A new dimension in plant protection

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ABSTRACT

In the present investigations it is proved that the inoculation of multifunctional microbial formulation to the soil improves soil quality, soil health, plant health, growth, yield, and quality of a broad spectrum of crops reducing chemical fertilizer and pesticide input. These microbial populations consist of selected species including plant growth promoting rhizo-bacteria, N2-fixing bacteria, Phosphate solubilizers, phytohormone producers, plant disease suppressive bacteria and fungi. To make it very simple a different dimension as 'Bio Protectant" is given in this presentation to the collective synergistic effects of beneficial microbes stimulating soil, plant and environmental health reducing chemical fertilizer and pesticide application. A healthy plant does not require unwanted, poisonous chemical pesticides. Review of literature is focused in multiple ways on the growth promoting ability along with the biological activity of beneficial microorganisms. The present SumaGrow-F2 formulation contains multi-functional Rhizobium species, *Pseudomonas* spp., *Bacillus*, and *Trichoderma* spp. The recorded novel benefits of SumaGrow-F2 formulation in the Green House and Field are discussed here as microbes increasing plant health, soil health, and root health of a broad spectrum of crops. Not only can it eliminate almost all insect infestations and reduce fungal or bacterial infection, it also creates a healthier environment for plant growth. The result is healthy crops with a corresponding increase in the yield of fruits or vegetables or grains. Modern trend is to look for inspiring intelligent multi-functional microbial Plant protectant or bio protectant for sustainable agriculture.

Key words: Multi-functional, polymicrobial, plant growth promoters, microbial plant protectant, bio protectant

INTRODUCTION

Excessive and inappropriate use of agrochemicals has undeniably resulted in negative and sometimes irreparable effects on soil health and the environment. In an effort to increase crop yield, soils have been fertilized heavily with chemical fertilizer when hybrid varieties were introduced for increased yield. Degraded soils and groundwater pollution caused by chemical leaching have resulted in lands becoming unproductive in the long run. In the same way, reliance on chemical pesticides to manage pest problems has aggravated environmental ruins. Pesticide residues raise food safety concerns among domestic consumers and pose trade impediments for export crops. While chemical inputs have raised agriculture's productivity levels, the benefits, however, are short-lived. As such, farmers and consumers are now in search of safe, secure, and sustainable alternatives to agrochemicals and would provide them safe and substantial amount of food without harm to the environment.

In the present report a new dimension is given to view the effective action of beneficial soil microbes with multiple functions as bio Protectant. Many of the beneficial microbes have been reorganized using molecular tools. There are different genera of Rhizobium isolated from different region as fast growing, slow growing, photosynthetic,, growth promoting, disease suppressing and degraders of toxic pollutants. There are wide exposure given to both chemical and biological control of serious pests/ plant diseases upon which a target with full of scope is easy to design and incorporate in to practice with new dimension for a broad spectrum of crops. In the earlier approach only the pathogen /pest controlling effect was highlighted but there are many other beneficial effects like Increase in Plant health, increased Brix value, increased host resistance, shelf life of end product, increased root and soil health, etc., that have not been added to the general health of the plant which is very important for reducing pest/disease attack. In the present report the focus is to the best of our knowledge how we can use the natural beneficial soil microbes for effective crop cultivation minimizing conventional fertilizers and pesticide input. Keeping in mind the plant health, root health and soil health for the best management of Crop cultivation, the multiple functions of soil microbes are probed into for nitrogen fixation, phosphate and potash solubilization, nutrient cycling, stimulators of phytohormone production and finally induction of host resistance against serious pests and diseases.

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MATERIALS AND METHODS

Isolation, characterization and molecular identification of the microbes consisting numerically predominant bacteria were made from the root nodules of a number of leguminous plants, soil and rhizosphere were carried out in Michigan State University.

Multifunctional microbial formulation: Multi functional microbial formulationSumaGrow-F2 with 14 bacterial and 7 Trichoderma strains individually mass multiplied (sequence of 10 ml test tube broth subsequently inoculated in to75ml and finally 1 liter TMY broth) and finally centrifuged and pelletted cultures were re-suspended in 12% Humic acid carrier (pH7) to yield 10^{17}cells/ml. All the operations were carried out under aseptic condition. Various other preliminary analysis, including microscopic examination, plate counts, most probable number (MPN) plant infection tests were carried out to meet quality standards (Reddy *et al*., 2007). A variety of factors combine to create “quality” in a poly microbial formulation. These include large cell numbers of superior microbial strains, free from significant contamination, a formulation that is effective and easy to apply with adequate shelf life, and proper carrier (Humic acid 12% with pH 7). Evaluation of inoculants quality by enumerating the viable cells present in the formulation is usually an accurate index of inoculant potential and shelf life for achieving the effective inoculants for enhanced productivity. Viability test was carried out at monthly intervals until one year. In addition to the laboratory construction of SumaGrow-F2 in MSU (Reddy and Lalithakumari, 2009) Bio Reactors 50 gallon capacity were used to produce large scale production for field trials in Bio Soil Enhancers Inc (BSEI) research laboratory. The constructed product was tested in the field at the rate of one gallon /acre for broad spectrum of crops (Chuck *et al.,* 2009). Always cell concentration (CFU) was maintained as trillion per ml.in all field studies conducted by BSEI Test plots.

Efficacy of SumaGrow-F2 relative to 12% Humic acid: All the Green House experiments were conducted using the Baccto potting soil rich in horticultural sphagnum, peat moss and perlite sufficient for initial seed germination. A randomized replicated design was followed to set up all the experiments using SG-F2 as the inoculant. Two split applications of the SG-F2 liquid formulation (described above with 10^{10}cfu per ml) were given per each pot (12”X12”X12”) during the crop period. The first application was given at the time of sowing as seed /soil treatment and the second application was given one month after the first application. The experiment was set up to compare the efficacy of SG-F2 over humic acid (12%) alone with out any added microbes as control. Each with 4 replications were conducted in a randomized replicated design. Exogenous fertilizers or chemical pesticides were not added to any of the three treatments during the crop period .The formulation was added in such a way that the final concentration of microbial cells will be 1×10^{10} / per pot (Hitbold *et al.*,1980). A broad spectrum of crops which includes cereals, vegetable crops, legumes, forage grasses and also bio fuel grasses like switch grasses were tested. Observations were made at monthly intervals during the entire crop period.

Efficacy of SumaGrow-F2 over conventional Fertilizer: In another green house experiment, the effect of the SG-F2 formulation was evaluated on selected crops Keeping 50% NPK 20-20-20 conventional fertilizer application as control. Plant height, total number of leaves, leaf area, leaf color, flowering time, fruiting time and number of fruits/weight of fruits/grains and shoot and root biomass were used as tools for evaluation of the multi functional microbial formulations under green house conditions. The test crops were also monitored for the incidence of pest and diseases during the crop period. Monthly observations were made up to the crop period. All data were subjected to statistical analysis (ANOVA 11). Field Experiments to evaluate SG-F2 multi functional poly microbial formulation (BSEI Inc.2009-2012).

Field Evaluation of SumaGrow-F2 over Conventional Fertilizer : Field experiments were set with 100% and 50% conventional fertilizer as control to evaluate Suma Grow only, Suma Grow combined with 50% fertilizer, 100%, and 50% fertilizer only as control using corn, soybean, and peanut as test crops. There were four treatments and four replications. Fertilizer 50% with SG-F2 treatment was compared over 50% and 100% conventional fertilizer. Fertilizer application was according to the existing Mississippi fertilizer recommendations. Suma Grow –F2 was applied at 1 gallon/acre in two split doses. Half a gallon of suma grow was applied at the time of sowing as side dressing for corn, soybean and peanut (T1 and T2). In T2 50% fertilizer was also applied as side dressing. The second application of SG-F2 for T1 and T2 was given one month after sowing. Sumagrow liquid was applied using the sprayer always focusing the base of the plant or soil.(intention is to make microbes reach the soil) Conventional fertilizer NPK 100% (T4) was also applied in two split doses. The test crops were Corn, Soybean and Peanut. All field experiments were conducted using RBD lay out with four replications.

RESULTS AND DISCUSSION

SumaGrow-F2 (hear after mentioned as SG-F2) has been selected to focus green technology by reducing chemical pesticides and fertilizers. The product is highlighted here with more evidences through field trials and greenhouse studies (Michigan State University, 2007-2009). Though these trials include a broad spectrum of crops such as corn, soybeans, cotton, rice, energy cane, biofuel grass, forage grass, oil palms, tomatoes, millets, oilseeds, fruits, vegetables, banana, coconut, lime, coffee, tea, areca nut, rubber, flower, spices, condiments, herbs, lawns, ornaments, trees etc., only selected crops are discussed here. The results showed a distinct increase in plant height with SG-F2 over the 12% humicacid. The mean weight of tomato was increased by 88% and fruit yield has been increased by 400% in SG-F2 as compared to the 12% humicacid control. The okra yield increased by 258.4% while rice yield increased by 301%. With rice, SG-F2 treated plants showed an increase in seedling vigor, plant height, number of tillers and their carry over effect on grain yield. All legumes tested showed early flowering, fruiting and good root nodulation. The powdery mildew disease infection was observed in squash. Importantly, the mildew infection was less in SG-F2 treated squash while in the 12% humicacid, the mildew affected plant totally wilted.

The conventional fertilizer was reduced to 50% and compared with SG-F2 combined with 50% fertilizer. SumaGrow-F2 only (T1) and SG-F2 with 50% NPK 20:20:20 (T3) were assessed over fertilizer only NPK50% (T2) control and the results are presented in Table 2. Various parameters taken here are plant height, chlorophyll content and total yield. The SG-F2 treated corn plants were tall and green with broad and long leaves. Interesting observations are the development of more number of brace roots in the SG-F2 treated plants (results not included) developing into the soil forming more silt roots. Further SG-F2 treated plants showed an early tassel and silk emergence. The cobs (earheads) matured 20 days before the plant which received 50% NPK. In fertilizer only treatment the brace roots were still suspending around the nodes above ground not penetrating into soil. The fertilizer treated plants further showed a higher incidence of spider mite attack when compared to the SG-F2 treatment with and without 50% fertilizer. Production of both tassel and silk were delayed in the fertilizer treated control and there was only one cob per plant while there were two cobs/plant in all the replications in SG-F2 with and without 50% fertilizer. The ear heads were completely filled confirming 100% total fertilization of the ear head. Like corn, soybean also exhibited distinct increase in plant height (Table 2). The vegetative stage showed a bushy canopy of green and big sized leaves.

Table 1. Comparison of SumaGrow-F2 (SG-F2) formulation with 12% Humic acid pH 7 (carrier)

Crop	Plant Height (cm) SG-F2	Plant Height (cm) 12%Humic acid	Yield (g) SG-F2	Yield (g) 12% Humic acid
Corn	142	101	292*	201
Sorghum	74	49	175*	120
Rice	65	55	20.9*	5.2
Tomato	77	66	1900*	380
Soybeans	168	98	11.6*	5.1
Pea	45	33	14.0*	7.5
Okra	130	98	138.7*	38.7
Peanut	42	35	21.6*	6.5
Pea purple hull	61	40	14.8*	10.8
Garden beans	135	102	48.6*	23.5
Wonder bush beans	89	65	72.9*	35.6
Squash	57	36	650*	0**

*Significant P=0.22 ** no squash matured

Early flowering was observed in the SG-F2 treated soybean plants. More interestingly significant increase in pod formation was observed in SG-F2 treated plants when compared to the fertilizer treated plants. This was equally matched with the several hundreds of nodules observed in the roots of SG-F2 treated plants. Diverse root nodules of different shapes and sizes were observed. The length of the primary and the lateral roots were more in SG-F2 treated soybeans. Total number of pods and the total yield are significantly high in the SumaGrow-F2 treated plants and are correlated to the numerous nodules present in both primary and secondary roots. In soybeans mildew spots were totally absent in the SG-F2 treatment, while the fertilizer 50% treated plants showed severe infection. Number of main-stem nodes and biomass increased in SG-F2 treatment with increased potential for early flowering and therefore longer reproductive period. Sumagrow-F2 treated soybean showed stronger stalk, healthier plants to gain resistance to both biological and environmental stress. In general nematode infection was not observed in all treatments.

Garden bean was continuously tested withSumaGrow-F2 formulation as it was very simple that it could be infected by a wide range of nodule forming bacteria. From the beginning of many trials the garden bean showed significant growth increase when compared to the fertilizer treated plants. The chlorophyll content of the SumaGrow-F2 treated plants were more than the fertilizer control. Photosynthesis is directly correlated to chlorophyll content. Here again early flowering and fruiting was observed. Each plant developed more number of side shoots giving a bushy appearance to the plants. The beans harvested from SG-F2 treatment and SG-F2 with 50% fertilizer treatment were long, and tender when compared to a short sized beans in the 50% fertilizer control. Significant diverse root nodules are correlated to the significant growth, pod production and total yield of theSumaGrow-F2 treated beans. The maturity of the pods reached in more than 25 days before the control plants. The leaf size was broad and greener than the fertilized plant. Nodules were observed in the fertilizer treated plants but only in the later stages of growth and were very less when compared to SG- F2 treatment.

Tomato is another most important vegetable crop in the world. Tomato plants irrespective of the variety showed a distinct growth and yield when compared to SG-F2 only and fertilizer 50% control (Table 2). Repeated experiments with tomato (greenhouse) showed significant increase in growth, number of fruits/plant and also the total weight. One of the very interesting observations is the bunches of flowers produced in the SumaGrow-F2 treated plants when compared to NPK 50% control. The flowers in theSumaGrow-F2 treatment were multi petaled open and showy when compared to control. The plants treated with 50% NPK (20:20:20) the growth initially was rapid but later on the leaves started turning purple in color, which is a typical deficiency symptom of the tomato plant for want of essential nutrient. This resulted in poor yield in fertilizer treated plants. The size of the fruit and the numbers also reduced in the fertilizer control. On the other hand SG-F2 treatment with fertilizer

Table 2. Comparison of the crop productivity of selected crops with T1. SumaGrow- F2 only, T2.NPK (20-20-20)50% only and T3. SumaGrow-F2 with NPK 50% (20-20-20)

Crops	**Plant height(cm)****			**Chlorophyll content****			**Total yield (g/plant)****		
CROP	T1	T2	T3	T1	T2	T3	T1	T2	T3
CORN	228.6*	143.0	244.5*	40.3	33.8	47.4	384.9*	119	563*
SOYBEAN	96.5	101.6	106.7	42	40	47	71.2*	44.4	71*
GARDEN BEAN	211.6*	264.2*	138.4	39.6	35.2	46.13	299*	192.8	504.5*
TOMATO	80.0	79.2	106.7	42	34	47	400*	140	720*
CLOVER	58.9	45.7	60.2	43.1	37.3	46.7	133*	107	159*

** Mean of 4 replications* significant, P = 0.022 Clover=Shoot biomass

50% NPK showed significant increase in the total yield over 50% conventional fertilizer though the height of the plants were almost the same. Chuck Grantham (2012) reported (personal communication) his Celebrity tomato treated with 50% conventional fertilizer with SG-F2 yielded approximately 20 lbs per plant when compared to chemical fertilized tomato yield was on an average 15lbs.Fruit size on SG-F2 treated plants was two and three ozs, larger than conventional treatment. Reducing fertilizer when using products containing SG-F2 is a pivotal benefit for producers-lowering input costs and reducing the risk of over fertilization. Gravel *et al.* (2007) have reported the growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* and *Trichoderma atroviride*. Several reports on the exciting yield performance of tomato with the present formulation therefore confirmed to the complementary action of the multi functional microbes.

Clover: SG-F2 treatment (T1) and SG-F2 with fertilizer (T3) showed green leaves and resulted in a fast growth with increased leaf size and chlorophyll content. The results of clover showed dramatic differences among the treatments T1, T2 and T3. The plant height of SG-F2 only and SG-F2 with 50% conventional fertilizer was 58.9cm and 60.2 cm, respectively, while the fertilizer treated plant height was only 45.7cm. The SG-F2 treated plants showed 3-5 branches with more number of large sized leaves. SG-F2 treated clover plants were tall, branched and with broader and green leaves and appeared healthier. The results in Table 2 showed clearly the significant increase in the plant height, chlorophyll content and total yield. More interestingly, the development of root nodules was numerous. Several hundreds of root nodules were observed inSumaGrow-F2 treated clover plants, when compared to root nodules in the fertilizer treated plants. The shape of the nodules were mostly white and slender in fertilizer treated clover but in SumaGrow-F2 treated plants both white and another pinkish finger shape or fan shaped nodules were observed. Plants with increased nodules showed increased shoot biomass. The fertilizer treatment like in tomato showed an initial growth but during the later stages the color of the leaves was slightly yellowish when compared to the other treatments. Less number of root nodules was observed. Field trials conducted to validate the functional quality of SumaGrow–F2 on selective crops confirmed the Green House results.

The results in Table 3 showed a significant and remarkable increase in the total yield of sweet corn (G-90) directly related to the number of cobs, length of the cobs, and kernel rows. The plant height in all the treatments did not show distinct variation but the total yield was more in T2 with SumaGrow +50% NPK. In addition, number of brace roots observed was more in the SG-F2 combined with 50% conventional fertilizer. The brace roots penetrated the soil and increased the root density. Increased brix value is another important credit added to SumaGrow treatment combined with 50% fertilizer. Head smut causing disfiguring galls on the ears of corn was observed in 100% fertilizer treated plants. Even insects like corn ear worms caused greater damage to corn in 100% fertilizer treated plots. The

Table 3. Field studies with Sweet Corn G-90 on the efficacy of SumaGrow-F2 with conventional fertilizer (100% and 50%)

Group	Treatment(s)	Height (cm)	No. of Cobs	Rows/Cob	BRIX	Cob Length (cm)	Total Yield (lb)
T1	SumaGrow-F2 only	194.0	39	16	8.6	21.6	19.4
T2	SumaGrow–F2+ 50% NPK*	199.4	78	16	9.8	22.3	40.1*
T3	50% NPK only	200.7	47	16	8.0	23.4	21.2
T4	100% NPK only**	203.2	53	14	8.4	21.6	24.7

No diseases observed. Corn borer attack was more in 100% and 50% conventional fertilizer treatment. Few corn Head smuts were observed in 100% fertilizer plots.
*The two-tailed P value for yield equals 0.0003 for groups T2 and T3. By conventional criteria, this difference is considered to be statistically significant; **The two-tailed P value for yield equals 0.0016 for groups T2 and T4. By conventional criteria, this difference is considered to be statistically significant.

high brix is nothing but the mineral density which reduced the pest and disease attack. Fungal leaf blights and rust were recorded during the late stages of corn but the disease intensity was more in 100% fertilizer treatment. The grain fill is another important and interesting observation in SG-F2 treatment with 50% fertilizer. The success of pollination is due to the pollen density as a result of high brix. The effect of SumaGrow-F2 is note worthy on corn because recent research shows that corn is high in antioxidants and carotenoids that are associated with health of human.

Peanut: Peanut (Table 4) also showed a significant increase in yield when SG-F2 was combined with 50% conventional fertilizer. SG-F2 treatment alone showed an increase in the 1000 pod weight over the 100% and 50% conventional fertilizer. Number of root nodules (active) was more in SG-F2 with 50% conventional fertilizer (T2) and SG-F2 (T1) only treated plants. The brix value is also more in SumaGrow-F2 only and SG-F2 with 50% conventional fertilizer treatments. Since SG-F2 is ecofriendly, it attracted more natural pollinators. Natural cross pollination resulted in increased pods with three to five seeds that develop underground. Flowering continued over a long period and pods were fully matured in SG-F2 with 50% conventional fertilizer. Gynophores remained strong under the ground in SG-F2 with 50% fertilizer and Suma Grow only treatments, while in 100% fertilizer treated plots the pods were separated in the soil due to peg rotting and the resulting loose pods have been lost during the harvest causing reduction in pod yield.

Table 5. Field Studies on the efficacy of SumaGrow-F2 with conventional fertilizer (100% and 50%); PK.Soybean – Asgrow 5606

Group	Treatment	Plant Height (cm)	Number of Pods/Plant	Total Yield (lb)
T1	SumaGrow-F2	91.7	214	6.3
T2	SumaGrow-F2 + 50% NPK*	98.0	377*	13.2
T3	50% NPK only	94.9	307	7.2
T4	100% NPK only	100.0	346	11.4

In 100% and 50% NPK treated plots, plants were green until harvest. In SumaGrow-F2 treated plants, leaves had turned yellow and the pods mostly dried and ready to harvest; No pests and diseases were observed during crop period.-No pesticides sprayed.
*The two-tailed P value for yield equals 0.0003 for groups T2 and T3. By conventional criteria, this difference is considered to be statistically significant.

Soybean: Soybean variety Asgrow 5606 in the field trial showed a significant increase in yield when SumaGrow-F2 was applied with 50% conventional fertilizer. Like Peanuts the number of pods was more in SG-F2 combined with 50% conventional fertilizer. Roots showed a rich branching in SG-F2 with 50% conventional fertilizers when compared to 100% fertilizers. Soybean is one of the biggest national crops. The stalk strength was more in Sumagrow-F2 treatment with 50% conventional fertilizers. This gives sustainable long lasting disease protection with improved profit potential. The beneficial microbes in SumaGrow-F2 boost plant health and disease suppression. Number of lateral branches was more with more number of pods in SG-F2 treatment with 50% fertilizers.

Maximum canopy leads to increased photosynthesis number of main stem nodes and biomass increase which is potential for early flowering and therefore longer productive period. The highest yield was observed in SG-F2 treatment with 50% fertilizers. Thus both Green house and Field trials observations clearly indicate that SumaGrow-F2 has the potential to provide significant commercial benefits through its ability to enhance soil health, plant health and increased crop yield with high brix. The product thus could be projected as a potential crop protectant reducing pest and diseases with multiple added benefits. The overall observations both in green house and field studies confirmed the ability of the present formulation utilizing functionally potential and beneficial soil microorganisms with synergistic and complementary characters for a sustainable agriculture reducing fertilizers and pesticides. Both in green house and Field experiments it was expected that leguminous plants which support symbiotic nitrogen fixation such as soybean, garden bean, wonder bush bean, pea and other legumes would give better results with SG-F2 formulation. But, it is noteworthy that even non-leguminous plants such as tomato, eggplant, zucchini, squash, rice, corn and sorghum, which are not associated with symbiotic nitrogen fixation, showed significant growth response. The present results clearly indicated that the beneficial microbes in SG-F2 contributed plant growth of broad spectrum of crops confirming the plant health and growth promoting abilities. These microbes are associated with the rhizosphere which is an important soil ecological environment for plant microbe interactions. Symbiotic nitrogen fixing bacteria include the *Rhizobium, Azorhizobium* and *Sinorhizobium* are present in the SG-F2 (Reddy and Lalithakumari, 2009). Free living nitrogen fixing bacteria like *Enterobacter, Burkholderia, Pseudomonas* and *Azospirillum* in the SumaGrow-F2 could have colonized the roots of grain crops efficiently contributing plant growth promotion through nitrogen fixation, phosphate soubilization and disease suppression. Bevivino *et al*. (1998) and Hayat *et al*. (2010) have reported the effect of both symbiotic and free living microbes in plant health and growth promotion. Bai *et al*. (2003) reported enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. These beneficial bacteria and *Trichoderma* in the present formulation promote the plant growth and yield by fixing nitrogen, solubilizing phosphate to available form, facilitating the uptake of plant nutrients from the soil, synthesizing growth stimulating growth hormones or compounds, and lessening or preventing the plants from disease through induction of host resistance. Solubilization and mineralization of nutrients particularly mineral phosphate reported in the present research supported by many researchers (de Freitas and Germida, 1991; Richardson, 2001). Yanni *et al.* (1997) and Afzal and Asghari (2008) reported successful association of rhizobia with cereal roots. Beneficial effect of inoculation with free living bacteria in both greenhouse and field conditions in graminaceous plants have been reported by many workers (Hegazi *et al*., 1998; Kennedy *et al*., 2004) in support of the present observation. Non-rhizobial bacteria synergistically act with rhizobia and enhance nodulation and grain yield possibly by indoleacetic acid (IAA) production, phosphate solubilization, nitrogen fixation, siderophore production etc. (Mishra *et al*., 2009; Rajendran *et al*., 2008).

Present field results appear to validate our hypothesis that enhanced plant growth and productivity can be obtained in a broad spectrum of crops when grown with microbial formulations containing microbes representing several different complementary functional groups (Reddy and Lalithakumari, 2009). It is also remarkable that the growth response and productivity with high Brix in corn, soybean and peanuts the most important food crops worldwide was quite encouraging and suggest that non-N2 fixers also give a growth response by producing metabolites/micronutrients that boost plant growth.(Balachandar *et al*., 2007). For example, it has earlier been reported that association of *Pseudomonas sp.* and *Trichoderma sp*. with cereal crops such as rice will result in increased productivity (Mishra *et al*., 2006). *Pseudomonads* *and Trichoderma* not only act as biocontrol agents but also produce metabolites that enhance plant growth (Yedidia *et al*., 2001). It is also possible that other free living N2-fixers such as *Paenibacillus and Burkholderia* might be contributing in part by providing fixed nitrogen to the plant (Balachandar*et al*., 2007*). Rhizobia* (*including Rhizobium, Bradyrhizobium, Mesorhizobium*, and *Sinorhizobium*) are generally regarded as microbial symbiotic partners of legumes and are mainly known for their role in the formation of nitrogen-fixing nodules (Antoun and Prévost, 2005). These bacteria can equally produce plant growth regulators and solubilize organic and inorganic phosphates(Reddy and Lalithakumari, 2009.) that would have a role in their plant growth promoting activities (Antoun *et al*., 1998). In addition to their plant growth promoting effects, *Rhizobium spp.*have been increasingly associated with disease suppressive effects in the present research. Garden bean and squash plants showed resistance to powdery mildew while the untreated squash wilted totally (Lalithakumari and Reddy, 2009). Recent literature (Elbadry *et al*., 2006; Huang *et al*., 2007) confirmed that *Rhizobium* spp. have been reported to be associated with disease suppression. Chung *et al.* (2008) reported the suppression of soil-borne pathogen of cucumber and pepper by *Bacillus subtilis*. Golami *et al.* (2009) further supported the effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of corn. Harish *et al.* (2009a) have reported the induction of disease related proteins by mixture of plant growth promoting bacteria against bunchy top of Banana virus. Similar reports have been made by Zehnder *et al*. (2001) on the induction of host resistance in tobacco against blue mold disease by plant growth promoting bacteria, Niranjan *et al*. (2003) on plant growth promoting microorganisms causing suppression of downy mildew in pearl millet. Silva *et al*. (2004) also supports the rhizobacterial induction of systemic resistance in tomato plants through non specific protection and increase in enzyme activity. Further, Zhang *et al.* (2010) reported on the control of *Phytophthora* blight of squash by the plant growth promoting In addition to their plant growth promoting effects, *Rhizobium spp*. have been increasingly associated with disease suppressive effects in the recent literature (Elbadry *et al*., 2006; Huang *et al*., 2007; Huang and Erickson, 2007; Siddiqui *et al*., 2007; Dutta and Podile 2010), *Pseudomonads* and Enterobacteriaceae family (Hynes *et al*., 2008), *Bacillus* (Herman *et al*., 2008) and *Azospirillum* (Russo *et al*.,2008).

Many Rhizobium spp. have been studied with regard to their potential mode of action in directly inhibiting plant pathogen growth, mainly with regard to the extracellular compounds that they produce. Another interesting observation in the present research is that soybean and squash exhibited resistance to powdery mildew when treated with the multifunctional microbial formulation, which is attributed to induced systemic resistance (ISR) and increased Brix value. Similar results have been demonstrated for powdery mildew caused by *Blumeria graminis f. sp. hordei* (Heitefuss, 2001), take-all caused by *Gaeumannomyces graminis var. tritici* (Khaosaad *et al*., 2007), leaf spot caused by *Pseudomonas syringae* (Ramos Solano *et al*., 2008) and root rot caused by *Fusarium culmorum* (Waller *et al*. 2005). Nandakumar *et al*. (2001) have reported the induction of systemic resistance in rice against sheath blight disease by plant growth promoting bacteria. The multiple function of the present formulation has also been validated through field trials and through private farmers and researchers who used the SG-F2. Among biocontrol bacteria, *Pseudomonas spp*. have been broadly studied for their ability to reduce the development of various soil-borne plant pathogens (Carisse *et al*., 2003).

Numerous modes of action for Pseudomonas spp. have been reported, including the production of different antimicrobial compounds and induction of the plant defense mechanisms (Ramamoorthy *et al*., 2002a, 2002b). In addition to their beneficial effect on the development of plants in the presence of pathogens, *Pseudomonas spp.*were also shown to improve plant growth in absence of pathogens. These results are in support of the healthy plants observed in the present research treated with SumaGrow-F2, which include *Pseudomonas fluorescens.*

Table 6. Field Studies in Peanuts on the efficacy of SumaGrow-F2 with conventional fertilizer (100% and 50%)NPK.

Group	Treatment	Brix	Number of Peanuts/Plant	Number of Root Nodules	Weight for 1000 pods (g)	Total Yield (lb)
T1	SumaGrow-F2	16.0	54	66	1670	48.1
T2	SumaGrow-F2 + 50% NPK*	15.0	60	59	1703	64.7
T3	50% NPK only	12.6	52	28	1609	50.2
T4	100% NPK only	14.4	56	19	1635	51.6

No leaf spots were observed. No nematode infection-no pesticides sprayed. More root nodules were observed in SumaGrow-F2 treated with and without 50% conventional fertilizer (5-20-20). Totally filled nuts were more in SumaGrow and SumaGrow-F2 with 50% conventional fertilizer treatments.

*The two-tailed P value for yield equals 0.009 for groups T2 and T3. By conventional criteria, this difference is considered to be statistically significant.

In addition to nitrogen, phosphorous made available by phosphate-solubilizing bacteria in SumaGrow-F2 may also be contributing to increased growth of cereals and vegetable with high Brix. More number of microorganisms in SG-F2 are acid producers (Reddy and Lalithakumari, 2009) and they are possibly presumed to play a major role in solubilizing phosphate, potash and other minerals and this could be an additional reason for increased yield in a broad spectrum of crops. More number of brace roots in corn growing downwards into soil and proliferate to fine root hairs at the time of tassel and silk production clearly indicated the synchronization of more nutrient uptake during the reproductive stage of the crop. The microbes in the formulation presumed to colonize the fine roots at the top surface of the plant or form endophytic association or else interacts with other microbes in the rhizosphere or phyllosphere, thereby influencing the observed plant growth, increased yield with high brix. The root system of all the tested crops (soybean,tomato,garden bean, clover, etc.) in the present report uniformly showed profuse branching indicating the possible uptake of plant nutrients causing rapid growth and maturity.

While microbial inoculants are applied to improve plant nutrition, they can also be used to promote plant growth by stimulating plant hormone production (Sundara *et al.*, 2002; Bashan *et al.*, 2004).The distinct plant height of corn, garden beans, soybean, tomato, okra, eggplant and clover with early flowering is the promising indication of the growth hormone production of the potential microbes in the present formulation. Bunches of large flowers (plate not included) where produced in mountain fresh tomato variety due to SumaGrow-F2 treatment. Thus, in the present research the overall results of healthier and larger plant with enhanced yield were related to the multifunctional microbes in SG-F2 formulation. The results of greenhouse trials have consistently showed enhanced growth and yield in all the experiments including the studies on the comparison between conventional nitrogen and SG-F2. In all the tested crops where fertilizer NPK 20:20:20 at 50% and 100% was applied the plants could grow in the beginning utilizing the readily available nitrogen but once it is exhausted, with no nitrogen available further to synthesize amino acid, a slowing of protein synthesis resulted in low brix and as a result growth slowed and the plants were susceptible to Pest and diseases(Arden B Andersen 1989).

Trichoderma spp. in the present SG-F2 have been reported to enhance root nodule production and increase in growth in garden beans (Reddy and Lalithakumari, 2009). Harman (2000), Mathivanan *et al*. (2000) and Harman *et al*. (2004) documented similar abilities of *T. harzianum* to enhance nitrogen use efficiency in corn but provided no insight into the specific mechanisms involved. Altomare *et al*. (1999) indicated that *T. harzianum* or its culture filtrates were able to solubilize iron, manganese zinc and rock phosphorus under in vitro condition. Anusuya and Jayarajan (1998) demonstrated solubilization of P from both tricalcium phosphate and rock phosphate by *T. harzianum* and *T. viride*. In the present research also two species of Trichoderma showed solubilizationof tricalcium phosphate and over all, the presence of seven Trichoderma strains with antagonistic potential and phyto hormone production established a clean healthy soil niche protecting the plants from pest and disease reducing the use of pesticides.

Overall, beneficial microorganisms in the present formulation, SumaGrow-F2 has demonstrated multifaceted beneficial effects pertaining to increased plant growth, chlorophyll content, Brix, early flowering and maturity , enhanced root nodulation and disease free healthy plants due to the disease suppression or systemic acquired resistance or induction of host defensive mechanism with increased yield. This could allow the release of their full potential as multifaceted beneficial bio-inoculants for improved growth, health and yield of crop plants with reduced input of chemical fertilizer and pesticides.

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