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The Stimulatory Effects of Humic Substances and Microbial Inoculants on Cropping Performance of Guava (*Psidium guajava* L.) cv. Lalit in Meadow Orcharding System †

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Abstract: Foliar application of humic acid along with bio-inoculants in 4-years old inarched guava cv. Lalit in meadow orcharding system was carried out. Foliar application of humic acid @ 30 and 60 mL/L at bud burst to flowering stage and bio-inoculants (Phosphorus solubilizing bacteria (PSB) @ 10 mL/plant + *Azotobacter chroococcum* @ 10 g/plant, and PGPR @ 25 mL/plant + AM fungi @ 25 g/plant) in rhizosphere along with @ 90 and 80 percent of recommended dose of fertilizers (RDF) of NPK (360:740:200 g/tree) was carried out. Application of humic acid applied at 60 mL/L along with PSB and *A. chroococcum* at 10 mL/plant beside 80 percent of RDF-NPK inferred positive impact on growth traits throughout the winter season. This combination recorded vital increase in percent fruit set with reduced fruit drop. Fruit yield was 2.9 times higher over control. Fruit quality with this conjoint application also improved. Soil microflora recorded as actinomycetes, *A. chroococcum*, PSB and AM fungi were improved. This combination also exhibited significant increase in leaf N, P and K contents in meadow geometry plant-soil interface. Maximum cumulative variance of 97.9 percent in PCA based on Eigen value (>1) was recorded. Maximum total cumulative variance for vegetative growth characteristics, flowering and yield contributing traits in guava under meadow plantations were observed in PC4. Our findings emphasized the promising effects of humic acid and bio-inoculants on improvement of growth, nutrient profiling and biological activity at reduced application of NPK.

Keywords: bio-inoculants; rhizosphere; humic substances; crop geometry

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1. Introduction

Guava (*Psidium guajava* L.), the poor man's fruit is synonymous to apple of the tropics, is an appetizing, nutritionally valuable and remunerative fruit crop. The crop has excelled with relevance to favorable growing factors (productivity, hardiness and antioxidant content. The cultivar, Lalit is one of the most widespread and high-quality cultivars available for guava farmers. The crop detaches among other tropical fruits for its taste, mineral composition, high lycopene content and possibilities of consumption, attributes that guarantee preference for different consumer markets worldwide. Meadow orcharding is a new concept of guava cultivation managing dwarf tree canopy with modified pruning at highly closer spacing of 2 m (row to row) × 1.5 m (plant to plant), which gives a density of 3333 plants/ha. Traditional planting at 6 × 6 m spacing has exhibited issues of low levels of productivity because of massive tree cover. Meadow orcharding is an embellished technique of fruit cultivation with changed tree cover promoting accrued rate of chemical change that ends up in high yield per unit space. The orcharding system utilizes small and dwarf trees with modified canopy architecture and growth regulation by the training and pruning. This technique of planting has revolutionized guava trade which boosts productivity including the reduction in cost of production. Humic acid, the moist and dark complex fraction is the most active part of soil organic matter (SOM) which accounts 60 percent that is answerable for many advanced chemical reactions in soil [1]. The effects of humic acid compounds have been attributed to improvement in physical, chemical and biological conditions of the soil. The bio-inoculants are cost effective and are renewable supplier of nutrients and thrive with symbiotic association with microbial strains that enhance productivity by (i) stimulating the biological organic processes, (ii) promoting the solubilization of insoluble phosphates in soil, (iii) the activation of plant growth vitamins, and iv) channelization of alternative growth regulating substances needed essentially for plant growth and development [2]. The study therefore, was planned to synergistic bio-stimulatory action through humic acid substances and bio-inoculants in guava (*Psidium guajava* L.) on growth, nutrient profiling and biological activity under meadow plantations in Shiwalik foothills of Himachal Pradesh.

2. Materials and Methods

The study was carried out at Regional Horticultural Research and Training Station of Dr YS Parmar University of Horticulture and Forestry at Dhaulakuan, Sirmour, Himachal Pradesh, India (Geographical coordinates: 35.5° N latitude, 77.5° E longitude, 468 m above mean sea level). The area experienced sub-tropical, sub-montane and low hill climatic conditions. Trees were selected based on similarities in their growth pattern to minimize variability caused by differences in tree size. The experimental manipulations used were three rows north to south to optimize effective light interception with lower air temperature and humidity. Four years old inarched guava trees of cultivar 'Lalit', spaced at 2 m × 1.5 m under meadow planting system were selected which received all usual scientific horticultural care.

Different fertilizer inputs *viz*., humic acid, PSB, *A. chroococcum*, PGPR and AM fungi were included. Humic acid (foliar spray) supplemented at two levels during winter season at bud burst to flowering stage i.e., HA_{30}-30 mL/L and HA_{60}-60 mL/L. Microbial inoculants ware applied to the rhizosphere at B_1: PSB @10 mL/plant + *A. chroococcum* @ 10 g/plant, and B_2: PGPR @ 25 mL/plant + AM fungi @ 25 g/plant along with reduced levels of RDF of NPK (360:740:200 g/tree) i.e., 90 percent of RDF (NPK_{90}), 80 percent of RDF(NPK_{80}). The experiment was designed in randomized complete block design with 8 treatment combinations (T_1–T_8) along with a control (T_9) with three replications (three trees per treatment). The treatments comprised of combinations; $B_1HA_{30}NPK_{90}$, $B_2HA_{30}NPK_{90}$, $B_1HA_{60}NPK_{90}$, $B_2HA_{60}NPK_{90}$, $B_1HA_{30}NPK_{80}$, $B_2HA_{30}NPK_{80}$, $B_1HA_{60}NPK_{80}$, $B_2HA_{60}NPK_{80}$ and Control.

Vegetative growth traits including plant height and girth of guava trees were recorded during the month of November and means were compared using standard errors. The representative sample size of uniform and healthy one year old shoots from the current season's growth in all the four directions were selected randomly for measuring shoot growth at the end of growing season in the month of October and the net increase was expressed in centimetre (cm). The trunk circumference measured at 10 cm above the graft union using measuring tape was recorded. Length of flowering was calculated at the beginning of the vegetation period in the trees. In order to investigate the effect of treatments on fruit set and drop, and duration of flowering were counted. Fruit set was determined as the percent of fruits per total remaining flowers. Fruit yield of the trees was recorded on each commercial harvest in kg/tree. The tree trunk circumference and canopy volume were used to work out yield efficiency (calculated as a ratio of yield/ TCSA and yield/TCV). Fruit samples were harvested randomly from each tree at fully ripe stage (physiological maturity) based on fruit firmness in February-March. The harvested fruits were then utilized for analysing fruit quality traits. Total soluble solids (TSS) were evaluated at 25 ± 2 °C of all sampled fruits at consumer maturity with a hand refractometer in °Brix.

3. Results and Discussion

3.1. Growth Indices

Humic acid and bio-inoculants combinations improved vegetative growth attributes, flowering, fruit yield, soil quality and leaf nutrient content during winter season guava plantations. The data showed that growth characteristics including, plant height, trunk girth, canopy diameter and shoot growth had noticeable improvement with respect to the application of humic acid and bio-inoculants along with inorganic fertilizers when supplemented to the trees under meadow orcharding. Enhanced N and P uptake was also positively correlated due to humic acid application with better vegetative growth in plants [3]. Humic acid promotes plant growth by stimulating IAA activity, which increases H^+ pumping through plasma membrane, lowers cell wall pH, and initiates cell wall loosening and expansion process. Besides, the improved uptake of micronutrients with humic acid application enhanced plant growth traits [4]. Humic acid being major component of organic matter in soil increases the plant height through improving photosynthesis rate, respiration, root growth, soil fertility, and nutrient elements uptake.

Table 1. Growth traits influenced by humic acid and bio-inoculants in meadow guava cv. Lalit.

Treatment	Plant Height (cm)	Trunk Girth (cm)	Canopy Diameter (cm)	Shoot Growth (cm)
$B_1HA_{30}NPK_{90}$	219.1 ± 2.3 g	21.2 ± 2.3 h	214.2 ± 2.2 h	11.2 ± 2.2 g
$B_2HA_{30}NPK_{90}$	221.8 ± 2.8 e	24.2 ± 2.3 e	216.1 ± 2.4 f	13.1 ± 1.1 f
$B_1HA_{60}NPK_{90}$	234.2 ± 2.6 b	27.2 ± 2.3 b	218.3 ± 2.2 c	15.6 ± 0.9 c
$B_2HA_{60}NPK_{90}$	221.9 ± 2.6 e	22.9 ± 2.4 f	216.7 ± 2.0 e	13.5 ± 1.1 e
$B_1HA_{30}NPK_{80}$	223.9 ± 2.4 d	26.1 ± 2.2 d	219.2 ± 2.3 b	15.9 ± 1.3 b
$B_2HA_{30}NPK_{80}$	219.6 ± 2.3 f	21.9 ± 2.5 g	215.1 ± 2.2 g	13.13 ± 1.9 f
$B_1HA_{60}NPK_{80}$	235.3 ± 2.3 a	28.1 ± 2.3 a	220.3 ± 2.3 a	16.4 ± 1.1 a
$B_2HA_{60}NPK_{80}$	232.7 ± 2.4 c	26.7 ± 2.3 c	217.8 ± 2.6 d	15.1 ± 1.0 d
N:P:K (360:740:200)	205.9 ± 2.3 h	22.1 ± 2.6 g	195.5 ± 2.2 i	9.2 ± 1.2 h

HA_{30}, humic acid at 30 mL/L; HA_{60}, humic acid at 60 mL/L; bio-inoculants consortium (B_1) i.e., PSB at 10 mL/plant + *A. chroococcum* at 10 g/plant; bio-inoculants consortium (B_2) i.e., PGPR at 25 mL/plant + AM fungi at 25 g/plant. The values represent mean (±SEM) of three replicates. The values followed by the same letter within each column are not significantly different from each other according to DMRT ($p \leq 0.05$).

3.2. Flowering and Fruiting

Application of 80 percent NPK significantly induced flowering and fruiting behaviour of guava during the cropping period. Foliar sprays of humic acid application have several advantages for guava production. During winter season, the trees which were treated with $B_1HA_{60}NPK_{80}$ treatment combination recorded maximum increment in length of flowering shoot. Optimum level of nutrients such as NPK and hormones had significant effect on increasing gibberellins in roots, thus breaking bud dormancy which in turn increased flowering buds [5]. The results obtained on fruit set and drop indicated that there were statistical differences among different treatments applied (Table 2). When the treatment combinations were compared based on average fruit set, $B_1HA_{60}NPK_{80}$ combination had relatively higher percent of fruit set (64.8%) followed by $B_1HA_{60}NPK_{90}$, $B_1HA_{30}NPK_{80}$ and $B_2HA_{60}NPK_{80}$ with corresponding values of 63.8, 62.9 and 62.51 percent, respectively. Fruit drop had a tremendously decreased in guava trees (13.8%) when supplemented with $B_1HA_{60}NPK_{80}$ treatment combination, whereas, maximum fruit drop (21.2%) was noticed in control. Similarly, application of humic acid combined with *A chroococcum* might have improved the fruit yield (Table 3) due to the role of bacteria in colonizing the rhizosphere, root surface and superficial intercellular spaces of plants, improving nutrient cycling through N fixation [6]. The use of humic acid results in the increased yield by stimulating photosynthesis [7]. Besides, it might influence fruit yield through mineral solubilization and through atmospheric N fixation [8]. The improvement in plant growth and canopy via application of humic acid allows better light interception by the plant thus increasing yield [9]. The best yield efficiency indicated that the treatment of $B_1HA_{60}NPK_{80}$ was recorded as the highest (1.3 kg/m^2 TCSA) which was further followed by $B_2HA_{60}NPK_{90}$ (1.2 kg/cm^2 TSCA), $B_1HA_{30}NPK_{90}$ (1.2 kg/cm^2 TSCA), $B_1HA_{30}NPK_{80}$ (1.1 kg/cm^2 TCSA), whereas, it was least (0.9 kg/cm^2) in control. In guava trees highest FY/TCV (1.1 kg/m^3 TCV) was recorded in $B_1HA_{60}NPK_{80}$ and minimum FY/TCV (0.5 kg/m^3 TCV) in control (Table 3).

Table 2. Yield contributing traits of guava cv. Lalit under meadow plantation.

Treatment	Duration of Flowering (days)	Fruit Set (%)	Fruit Drop (%)
$B_1HA_{30}NPK_{90}$	40.5 ± 0.7 f	57.9 ± 1.0 h	18.1 ± 0.5 b
$B_2HA_{30}NPK_{90}$	42.3 ± 1.4 b	60.0 ± 0.8 f	16.8 ± 0.2 c
$B_1HA_{60}NPK_{90}$	41.2 ± 0.8 d	63.8 ± 0.7 b	14.6 ± 0.3 g
$B_2HA_{60}NPK_{90}$	41.7 ± 0.9 c	61.8 ± 0.8 e	16.1 ± 0.5 d
$B_1HA_{30}NPK_{80}$	40.6 ± 0.8 ef	62.9 ± 1.0 c	13.8 ± 0.1 h
$B_2HA_{30}NPK_{80}$	39.4 ± 1.4 g	59.0 ± 1.1 g	15.6 ± 0.4 e
$B_1HA_{60}NPK_{80}$	38.3 ± 0.6 h	64.8 ± 0.5 a	13.7 ± 0.2 h
$B_2HA_{60}NPK_{80}$	40.7 ± 1.1 e	62.5 ± 0.9 d	14.8 ± 0.4 f
N:P:K (360:740:200)	45.2 ± 1.3 a	47.9 ± 0.8 i	21.2 ± 0.8 a

HA_{30}, humic acid at 30 mL/L; $HA_{60,}$ humic acid at 60 mL/L; bio-inoculants consortium (B_1) i.e., PSB at 10 mL/plant + *A. chroococcum* at10 g/plant; bio-inoculants consortium (B_2) i.e., PGPR at 25 mL/plant + AM fungi at 25 g/plant.

Table 3. Fruit yield (FY) and yield efficiency of guava cv. Lalit under meadow plantation.

Treatment	Fruit Yield (kg/tree)	Yield Efficiency	
		FY/TCSA (kg/cm^2)	FY/TCV (kg/m^3)
$B_1HA_{30}NPK_{90}$	4.1 ± 0.1 h	1.2 ± 0.1 ab	0.7 ± 0.1 c
$B_2HA_{30}NPK_{90}$	4.3 ± 0.1 g	0.9 ± 0.1 c	0.8 ± 0.1 c
$B_1HA_{60}NPK_{90}$	5.5 ± 0.9 c	0.9 ± 0.1 c	0.9 ± 0.1 bc
$B_2HA_{60}NPK_{90}$	4.8 ± 0.1 e	1.2 ± 0.1 ab	0.8 ± 0.1 bc
$B_1HA_{30}NPK_{80}$	5.9 ± 0.1 b	1.1 ± 0.1 ab	1.0 ± 0.1 ab
$B_2HA_{30}NPK_{80}$	4.5 ± 0.1 f	1.0±0.1 bc	0.9 ± 0.1 c
$B_1HA_{60}NPK_{80}$	6.4 ± 0.1 a	1.3 ± 0.1 a	1.1 ± 0.1 a
$B_2HA_{60}NPK_{80}$	5.1 ± 0.1 d	1.1 ± 0.1 bc	0.8 ± 0.1 c
N:P:K (360:740:200)	2.2 ± 0.1 i	0.9 ± 0.1 c	0.5 ± 0.1 d

HA_{30}, humic acid at 30 mL/L; $HA_{60,}$ humic acid at 60 mL/L; bio-inoculants consortium (B_1) i.e., PSB at 10 mL/plant + *A. chroococcum* at 10 g/plant; bio-inoculants consortium (B_2) i.e., PGPR at 25 mL/plant + AM fungi at 25 g/plant. The values represent mean (±SE) of three replicates. The values followed by the same letter within each column are not significantly different from each other according to DMRT ($p \leq 0.05$).

3.3. Fruit Quality

It is evident that foliar sprays of humic acid and rhizosphere application of bio-inoculants aimed at gaining better response in guava trees. The physical parameters of fruits were improved with conjoint combinations humic acid and bio-inoculants which might be attributed to better vegetative growth, resulted due to higher accumulation and translocation of photosynthates (starch and carbohydrates) in developing fruits and thus, have increased fruit dimension and weight. The effect of humic acid on the enhancement of photosynthetic pigment accumulation and photosynthesis rate could explain the rise in fruit weight [10]. These quality traits were also on account of influential role of bio-inoculants which might have aided in higher N fixation and uptake, thereby stimulating the catalytic activity and number of enzymes in the physiological processes of plants and increased production of sugars and amino acids which in turn increased TSS, acidity, TSS: acid ratio, total sugars and ascorbic acid content of the fruits [11]. Increased fruit quality might be attributed to the involvement of NPK in various energy sources like amino acids and amino sugars which ascribed in converting complex substances into simple sugars and enhanced the metabolic activity of fruits which in turn resulted in increased fruit quality.

3.4. Post Harvest Soil Chemical Indicators

The significant effect was observed in nine different treatment interactions on soil pH and EC, but differences in pH were negligible. Different combinations of humic acid, bio-inoculants and NPK changed pH of the soil towards neutral. Moreover, the data presented on soil OC content indicated that it was significant due to varied treatment combinations supplemented. Maximum soil OC build up was observed with the inclusion of $B_1HA_{60}NPK_{80}$. Among the tested combinations, $B_1HA_{60}NPK_{80}$ showed maximum available macronutrients NPK in the rhizosphere. The decrease of pH value was attributed due to the production of organic acids *viz.,* oxaloacetic acid, aspartic acid and glutamic acid. However, the reason for the slight increase in pH due to bio-inoculants was probably due to the moderation or buffering brought about by them. The increase in pH, EC and OC levels in soil might be due to the breakdown of organic matter after the incorporation of both bio-inoculants and humic acid. Soil organic matter has been considered to be greatly responsible directly or indirectly for making the physical environment of soils favourable for growth of crops. *A. chroococcum* when applied, fixed the atmospheric nitrogen in soil due to its nitrogen fixing properties, while, PSB was involved in increasing the availability of phosphorus in soil due to its solubilizing properties and thus compensated the reduced dose of NP and thus, maintained better soil environment which ultimately reflected on fertility status of soil [12]. Humic acid is the main component of soil organic matter which influences the soil parameters, including nutrient solubility by forming complex forms with chemical compounds of humic materials.

3.5. Leaf Nutrients

The interpretation of leaf nutrient content to assess plant nutritional status of trees. To interpret the results of traditional chemical analysis of plant tissue for the assessment of the nutritional status of plants, the methods of critical level and sufficiency range are used frequently. In the present study, foliar sprays of humic acid had a significant effect on the amount leaf macronutrient concentration of the trees. Increase in leaf nutrients in different treatments receiving bio-inoculants suggested that they solubilized the available nutrient pool in the soil and thus improved the uptake of nutrient content [13], increased solubility on account of organic acids through microbial inoculation, increased root surface to volume and permeation of hypal pads patch beyond exploration by root hairs [11]. Humic acid increased nutrition intake especially leaf N, P, and K content which might be related to the improved root growth and enhanced permeability of plant membranes thereby increasing plant growth [14]. The positive effect of humic substances also increased P recovery when interfered with calcium phosphate precipitation [15].

3.6. PCA Studies

Principle Component Analysis (PCA) was also worked out to identify the effect of humic acid and bio-inoculants on the vegetative and yield related traits. It summed up the correlation between the factor and the two illustrated axis of vegetative growth characters (plant height, trunk girth, canopy diameter, shoot growth), flowering and fruiting characters (length of flowering shoot, number of flower buds per shoot, duration of flowering, fruit set, fruit drop) and yield contributing traits (Table 4). The first components that accounted for the highest total variance based on the *Eigen* value (>1) were found by PCA studies and showed 84.8% (PC1), 92.7% (PC2), 96.1% (PC3) and 97.9% of the cumulative variance. PC4 registered the highest overall cumulative variance among vegetative, generative and yield related traits of guava under meadow orcharding. The PCA studies incorporated the variables with values that are equal to or greater than two-third of the value of highest variable within each PC. PCA was also used to determine the efficacy of humic acid and bio-inoculants on soil chemical properties and leaf nutrients (data not shown). The first components that further accounted for the highest total variance based on the *Eigen* value (>1) were found by PCA studies and showed 67.3% (PC1), 81.1% (PC2), 89.1% (PC3) and 95.4% of the cumulative variance. The PCA biplots with original variables/ factors drawn as 'Eigen vectors' depicted the correlation between the factor and the two illustrated axis of soil chemical properties (pH, EC, available NPK); soil microbiological properties (actinomycetes, *A. chroococcum*, phosphorus solubilizing bacteria, AM fungi spores) and leaf nutrients (NPK). Furthermore, the maximum variance for PC1 was recorded as 67.3 percent, whereas, PC4 recorded the minimum variance (6.1%). Similar hypothesis on PCA were also documented in strawberry [16], pistachio nut [17] and apple [18,19].

Table 4. PCA of growth traits, flowering and yield contributing parameters under meadow guava.

Parameter	**Principal Component**							
	PC1	**PC2**	**PC3**	**PC4**				
Eigen value	13.6	1.3	0.6	0.3				
Variability (%)	84.8	7.9	3.4	1.8				
Cumulative variance (%)	84.8	92.7	96.1	97.9				
Variables	**Factor Loadings (Pattern Matrix)**				**Eigen Vectors**			
	F1	**F2**	**F3**	**F4**	**PC1**	**PC2**	**PC3**	**PC4**
Plant height	0.9	−0.2	0.0	0.3	0.3	−0.2	0.0	0.5
Trunk girth	0.8	−0.5	0.4	0.1	0.2	−0.4	0.5	0.1
Canopy Diameter	0.9	0.1	−0.3	0.0	0.3	0.1	−0.4	−0.1
Shoot growth	1.0	−0.2	0.1	−0.1	0.3	−0.2	0.1	−0.2
Duration of flowering	−0.8	−0.4	0.1	−0.1	−0.2	−0.4	0.1	−0.3
Fruit set	1.0	0.0	−0.2	0.0	0.3	0.0	−0.2	0.0
Fruit drop	−1.0	0.0	0.0	0.2	−0.3	0.0	0.0	0.3
Fruit yield	1.0	0.1	0.1	−0.1	0.3	0.0	0.1	−0.2
YE (TCSA basis)	0.5	0.8	0.4	0.1	0.1	0.7	0.5	0.2
YE (TCV basis)	1.0	0.2	0.1	−0.2	0.3	0.2	0.1	−0.4
Factor Score (Treatment-Wise)								
Treatment	**F1**	**F2**	**F3**	**F4**				
$B_1HA_{30}NPK_{90}$	−2.0	1.7	−0.5	0.7				
$B_2HA_{30}NPK_{90}$	−0.9	−1.0	−0.9	−0.3				
$B_1HA_{60}NPK_{90}$	2.7	−2.0	−0.5	0.2				
$B_2HA_{60}NPK_{90}$	0.1	1.0	−0.1	−0.2				
$B_1HA_{30}NPK_{80}$	2.7	0.2	0.5	−1.2				

$B_2HA_{30}NPK_{80}$	−0.8	1.0	−0.9	−0.2
$B_1HA_{60}NPK_{80}$	4.9	0.5	1.3	0.4
$B_2HA_{60}NPK_{80}$	1.9	−0.8	0.1	0.7
N:P:K (360:740:200)	−8.6	−0.8	1.0	0.1

YE, Yield efficiency; TCSA, trunk cross-sectional area; TCV, Tree canopy volume; PC1, Principal component-1; PC2, Principal component-2; PC3, Principal component-3; PC4, Principal component-4.

4. Conclusions

Foliar application of humic acid at 60 mL/ L at bud burst stage along with soil application of bio-inoculants (PSB @ 10 mL/plant+ *A. chroococcum* @ 10 g/plant) in rhizosphere zone along with 80 percent of RDF of NPK had positive and significant effects on morphometric, generative traits, flowering and fruiting behaviour. Fruit qualitative traits were also improved during winter season. The bio-organics application also improved chemical properties of soils. The reduction of 20 percent inorganic NPK fertilizers through inoculation of *A. chroococcum*, P-solubilizers and AM fungi was achieved which might be responsible for better cropping behaviour and productivity of guava under meadow plant-soil interface in winter season.

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