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Production of Plant Garbage Juice and Its Effects on Plant Growth, Antibacterial Activity and Soil Reconditioning

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Abstract

Plant Garbage Juice (PGJ) is a highly concentrated bio-molecular solution. It contains water and bio-molecules such as acetic acid, sugar, proteins, alcohol, as well several enzymes like protease, amylase, lipase, and papain. It is promoted as a multifunctional solution for agricultural and domestic needs. In this investigation, two types of PGJ solutions were prepared to observe their various effects. The first one was prepared with mixed vegetable peels (PGJ-1) and the second with citrus peels (PGJ-2). Both solutions were acidic in nature, pH and EC (Electrical Conductivity) were detected as 4.3, 5.3 in PGJ-1, and 3.3, 2.58 in PGJ-2, respectively. PGJ-1 solution was applied to *Aloe chinensis*, a dwarf cultivar, and the growth rate was found to be higher than the control. In treatment 2 (T-2) of PGJ-1, the 2:1000 solution with water showed the best results for plant growth activities. The leaves of *Aloe chinensis was found to* turned brown green to dark green and the leaf thickness increased from 3.1 mm to 12.6 mm. The trash enzymes of PGJ solutions (I and II) exhibited antibacterial action against gram-positive and gram-negative bacteria, especially against pathogenic drug-resistant *E*. *coli*. PGJ-1 solution showed the highest zone of inhibition of 16 mm comparable to commercial antibiotic disk. PGJ solutions that have a catalytic enzyme showed the soil reconditioning process, such as a reduction of soil salinity. The mean of 20 soil samples showed that PGJ decreased the pH from 7.78 to 7.43. Similarly, the mean EC value of 20 soil samples also decreased from 0.45 ms/cm to 0.15 ms/cm. The pH and EC values of an alkaline sample also decreased significantly from 8.05 to 7.31 and 1.85 to 0.12, respectively. The results determined indicate that PGJ is a cost-effective plant growth-promoting factor and an eco-friendly substitute for minimizing global threats to agriculture.

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Introduction

Plant Garbage Juice (PGJ) is a fermentation product produced by using waste products in the kitchen such as fruits and vegetable peels. It is also called Garbage Enzyme (GE) or Eco-enzyme or Bio-enzyme (Arun and Sivashanmugam 2016). The garbage enzyme is a complex organic substance of protein chains, organic acids, and mineral salts produced easily by simple fermentation of fruit and vegetable wastes in water mixed with brown sugar or molasses sugar at the ratio of 10:3:1 (Neupane and Khadka 2019). PGJ preparation revealed the presence of acetic acid, sugar, proteins, alcohol, and activated enzymes such as protease, amylase, lipase, and papain (Vama and Cherekar 2020). Waste from various fruits and vegetables displayed varying levels of enzyme and antibacterial activity. Eco-enzyme functions as an anti-bacterial, insecticidal, and anti-fungal agent (Vama and Cherekar 2020). This solution is claimed to have multipurpose uses for household and agricultural applications. It is reported to act as a cleanser and disinfects all surfaces in the household, fertilizer, soil conditioner, air purifier, natural pesticide, insect repellent, eliminates electromagnetic radiation, purifies groundwater, etc. Every day, large amounts of fruit and vegetable waste are generated as garbage in both households and industrial sources. This is one of the major reasons for environmental pollution. It is postulated that this Organic Solid Waste (OSW) will become a significant problem around the world by 2025 due to an increase in the generation of food waste of up to 44% (Arun and Sivashanmugam 2016). The conventional disposal of OSW causes serious environmental pollution and health risks for living things. It contains a significant amount of organic matter that eventually degrades to produce carbon dioxide and methane (Arun and Sivashanmugam 2016). Consequently, the production of PGJ could put these wastes to better use and protect the environment. It helps in solid waste management and additional application of PGJ may provide a cost-effective environment-friendly alternative product for many other purposes.

PGJ is a cost-effective material that has the potential to improve the soil texture, inhibit the growth of pathogens, enrich the nutrient quality of soil, and enhance the plant yield (Sarabhai and Arya 2019). It contains various living microorganisms having symbiotic associations with plants helping in their efficient growth by increasing plant enhancements (Singh et al. 2020). There are several natural products in PGJ used to improve soil fertility, such as animal excretory waste, eggshells, carbon ash, plant fibers, and agro-waste (Tong and Liu 2020). PGJ contains nutrients that help plant growth and give quick metabolic support. PGJ also enhances soil activity and decreases soil salinity which could put wastes to better use and protect the environment (Meena et al. 2016). Therefore, the application of this solution in plants, antimicrobial activity test, activity as a cleansing agent, and observation of the reconditioning ability of soil was carried out.

Materials and Method

The process of producing plant garbage juice known as garbage enzyme fully depends on the fermentation technology. For the fermenting procedure, the gathered fruits and vegetable samples were combined with 1 part molasses, 3 parts peels of the fruits and vegetables, and 10 parts water. In an airtight plastic container, the solution was incubated for three months. Initially, to release the gas produced by fermentation, the liquid was stirred each day with a glass or wooden rod. The solution was further mixed once in every two weeks in the second month and once in the middle of the third.

After fermenting the vegetable peels for three months, the PGJ solution was prepared. It was a highly concentrated liquid solution that contained several types of enzymes. So, it had to be diluted with water before applying to plants. The dilution ratio was 1:1000, 2:1000, 3:1000, and 4:1000 where 1, 2, 3, and 4 ml of filtered liquid were added to 1000 ml of water and named as T-1, T-2, T-3, and T-4, respectively. The PGJ was applied in two groups. Therefore, the application was divided into two groups as follows:

In group 1, three batches of *Aloe chinensis* (a dwarf cultivar) plants (each batch contains 10 plants) were selected for the experiment. They were irrigated with normal water for two months to observe their physiological change regularly. All dates were recorded after applying the prepared nutrient juice solution (PGJ) to make a comparative result. This treatment was continued for three months and the data were collected at 15 days interval.

In group 2, four batches of *Aloe chinensis* plants were selected for the experiment. Each batch contained 20 plants, four plants were marked as control, and sixteen plants were prepared for applying treatment at the concentrations of 1:1000, 2:1000, 3:1000, and 4:1000 ratio (with H2O in ml). In the control plant, normal water was used. The duration of this experiment was six months and compared data from two groups (experimental and control) were recorded on plant growth.

Antimicrobial testing was carried out on three samples using standard disk diffusion and agar well methods, following The National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The tests were conducted on Mueller Hinton agar plates. Entero-invasive *E. coli*-ATCC 25922 (EIEC), a particular kind of pathogenic aquatic bacteria, was selected for this investigation, collected from our previous study. As a control, five brand-name antibiotic discs (Biomaxima, Poland) and sanitizers were used. The antibacterial substances examined were: a) Ciprofloxacin (CIP-5), b) Vancomycin (VA-30), c) Linezolid (LNZ-30), d) Clindamycin (DA-2), e) Colistin Sulphate (CT-10), f) Sanitizer 70% ethanol (S), g) Mixed PGJ (M-1), h) Citrus peels PGE (C-1), i) Citrus + Lemon peels (CL-1).

The PGJ solution was applied to show the effect on soil salinity and nutrient delivery. Twenty soil samples were examined in this investigation. The PGJ solution was diluted with water at a ratio of 1:1000, and 250 ml of this diluted solution was applied to each soil sample once a week for six months. Two sets of observations were made: one pre- and one post-treatment. At the end of the treatment period, tests were conducted to measure pH, electrical conductivity (EC), and total dissolved solids (TDS) to assess soil salinity based on EC values. Initial soil samples were collected from the surface layer (0-15 cm depth) at the experimental site. Data of plant growth activities were analyzed by analysis of variance (One-way ANOVA) and presented as mean values ± standard error (SE) with considering statistically significant at P <0.05. A Tukey's multiple comparison test was used to distinguish the difference between performed treatments. Data were analyzed by using SPSS (version 18.0). The standard deviation for the analysis of soil pH and EC was calculated using Microsoft Excel 365, a widely used spreadsheet software.

Results and Discussion

The liquid quality of PGJ was assessed by observing its odor, color, turbidity and gas formation, confirming that it was suitable for further experimental application. Table 1 provides a detailed comparison of the physical characteristics between PGJ-1 (prepared from mixed vegetable peels) and PGJ-2 (prepared from citrus peels). The characteristics of PGJ 1 and 2 are given in Table 1.

The biological and physicochemical properties of PGJ were further analyzed in Table 2, which lists key parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS), and optical density (OD). The pH values of the PGJ solutions were acidic, with PGJ-1 (mixed vegetable peels) having a pH of 4.13 and PGJ-2 (citrus peels) having a slightly lower pH of 3.43, suggesting the presence of volatile organic acids, likely acetic acid. The electrical conductivity (EC) was higher in PGJ-1 (5.30 ms/cm) than in PGJ-2 (2.58 ms/cm), indicating a higher concentration of ionic compounds in the mixed vegetable peel preparation. The TDS values supported this observation, with PGJ-1 containing 2000 mg/l and PGJ-2 1344 mg/l, reflecting the higher organic matter content in the mixed vegetable waste. The optical density (OD) of PGJ-1 and PGJ-2 were 1.67 Abs and 1.52 Abs, respectively, indicating the presence of dissolved organic material in both PGJ preparations.

PGJ was applied to the soil of the plant to determine its effect on plant growth. Initially, normal tap water was applied for the first two months, however, no significant change was observed in the growth of the experimented plants. Even the leaves of the plants were turned dark green to brown-green under tap water treatment. However, after starting treatment with different concentrations of PGJ solution, a noticeable

improvement in plant growth, leaf colour, length, thickness, and weight occurred. Table 3 summarizes the observations between the control and experimental treatments with PGJ-1.

Characteristics of PGJ	$PGJ-1$	PGJ-2
рH	4.13	3.43
EC.	5.3	2.58
TDS	2000	1344
ΩD	1.67	1.52

Table 2. Characteristics of PGJ solution (PGJ-1 and PGJ-2).

Table 3. Comparison of plant growth, leaf length, thickness, and leaf weight between control and experimental samples with PGJ-1 treatment.

Plant growth was observed in 75% of the control treatment, 92% of T-1 (1:1000), 97% of T-2 (2:1000), 90% of T-3 (3:1000), and 85% of T-4 (4:1000). There were significant differences in the increase of length in experimental and control plants (p <0.05) (Table 4 and Fig. 1). Significant differences in leaf weight was also observed (p <0.05).

Table 4. Effects of PGJ-1 on leaf length, leaf thickness, and leaf weight (statistical analysis was done using an analysis of variance).

Treatment	Mean leaf length $(cm) \pm SE$	Mean leaf thickness $(mm) \pm SE$	Mean leaf weight (q) ± SE
$T-1$	14.5 ± 0.224	$10.42 + 0.089$	$26.9 + 0.690$
$T-2$	$16.7 + 0.153$ ^a	12.37 ± 0.079 ^a	$42.8 + 0.696a$
$T-3$	$12.2 + 0.291$ _{bc}	9.05 ± 0.045	$22.7 + 0.633$ bc
$T-4$	10.5 ± 0.167 ^{cd}	$8.45 \pm 0.109c$	$17.6 \pm 0.521c$
Control	$9.5 + 0.224$ d	$6.07 + 0.037$ d	$14.875 + 0.441$ ^d

From statistical analysis, p-values for all three tests are p <0.05, we reject the null hypothesis for each case. This means that the observed differences in leaf length, thickness, and weight are not due to chance and the treatments (T-1, T-2, T-3 and T-4) and the control group have a significant impact on these characteristics of the leaves.

Among the different treatments, T-2 (2:1000) showed the best result in plant growth, length, thickness, and weight (Table 4). The color of leaves also changed back from brown-green to dark green observed in the same treatment (Fig. 1). These findings demonstrated that PGJ has a pronounced positive effect on overall plant growth. It may have happened due to the metabolic activities in plants enhanced by the presence of various enzymes and nutrients produced during the fermentation process of PGJ.

Fig. 1. Responses of PGJ solution in plant growth. (a)+(b)-growth of treated *Aloe chinensis* plants; (c)+(d) difference of leaf color between control and PGJ solution treated plant, (e)+(f)-thickness of leaf in group-1, (g)+(h)-thickness of leaf in group-2, and (i)+(j)-growth level of control plant and PGJ solution treated plants.

The antibacterial properties of PGJ were evaluated through an antibiogram test against *E. coli*. As shown in Fig. 2, PGJ extracts demonstrated varying degrees of antibacterial activity. Among the tested samples, PGJ extracted from citrus peels exhibited the highest zone of inhibition, 16 mm, against *E. coli* which was resistant to Vancomycin (VA-30) and Linezolid (LNZ-30) antibiotics used for this experiment (Table 5). PGJ from mixed vegetable peels also exhibited activity, with a zone of inhibition of 10 mm. In contrast, the antibiotics such as VA-30, and LNZ-30 also showed resistance. The sanitizer (S) also exhibited strong antimicrobial activity, further validating the antimicrobial potential of PGJ.

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Fig. 2. Observations of microbial activity of PGJ solution against pathogenic *E*. *coli*. (a) The ager-well method for PGJ-2 solution, (b) the disc diffusion method for PGJ-1 and PGJ-2 solution, (c) The ager-well method for PGJ-1 solution.

The impact of PGJ on soil health was evaluated by measuring the soil pH and electrical conductivity (EC) before and after PGJ application. The results from 20 soil samples indicates that the average pH decreasing from 7.78 (before treatment) to 7.41 (after treatment) (Fig. 3). There is a general decrease in pH in most samples after treatment. The pH decreases by as much as 0.74 (from 8.05 to 7.31 in sample 6).

Table 5. Antibiogram test results (zone of inhibition in mm) in *E. coli***.**

Here: a) Ciprofloxacin (CIP-5), b) Vancomycin (VA-30), c) Linezolid (LNZ-30), d) Clindamycin (DA-2), e) Colistin Sulphate (CT-10), f) Sanitizer 70% ethanol (S), g) Mixed PGJ (M-1), h) Citrus peels PGE (C-1), i) Citrus + Lemon peels (CL-1).

The EC values also showed a marked reduction, from an average of 0.45 ms/cm to 0.15 ms/cm (Fig. 3), indicating a decrease in soil salinity. EC tends to decrease after treatment for most samples, with the largest decrease occurring in sample 6 (from 1.85 to 0.12 ms/cm), which may indicate a significant change in the sample's chemical

composition or environment. The pH tends to decrease moderately, but the EC decreases much more significantly, indicating a reduction in the ionic strength or conductivity of the samples after treatment.

In our control group mean value of pH and EC was recorded as 6.78 (After) to 7.05 (before) and EC 0.52 (After) to 0.53 (before). The data suggests that the treatment caused a reduction in both pH and EC in most samples, with some variability across individual samples. The overall trend is a decrease in both parameters after treatment.

These results indicate that this decrease in pH and EC can contribute to soil reconditioning, making the environment more conducive to plant growth. PGJ, with its rich enzymatic activity, possibly facilitated the breakdown of soil compounds, enhancing nutrient availability and lowering salinity.

Fig. 3. (a). Mean values of pH. Before observation: Mean values of pH 7.78 (20 samples) Standard deviation 0.3416. After observation: Mean values of pH 7.43 (20 samples) Standard deviation 0.34866, (b) Mean values of EC. Before observation: Mean values of EC 0.45 ms/cm (20 samples) Standard deviation 0.3528. After observation: Mean values of EC 0.15 ms/cm (20 samples) Standard deviation 0.05207.

In this experiment, we observed the plant growth activity by using a PGJ solution which is the first experimental observation in *Aloe chinensis*. Vama et al. (2020) used PGJ solution at a concentration of 1:1000 ratio for seedling development and found that the use of PGJ solution can enhance the development of seedlings. This finding is similar to our research work. We can develop a protocol for using PGJ solution as a plant growth substance. Our antimicrobial activity for the PGJ-1 solution showed a 10 mm zone of inhibition which is similar to the result obtained by Teh et al. (2014). They found an 11 mm zone of inhibition against *Escherichia coli* by using mixed vegetable peels plant garbage solution. Bulai et al. (2021) also used garbage enzyme solution from fruit peels for reconditioning soli. Meena et al. (2016) observed the change in biological and chemical properties in saline soil. In our experiment, we identified that this solution can help in decreasing the soil salinity and reconditioning the soil.

The results from this study essentially indicates that PGJ produced from fruit and vegetable waste, possesses beneficial effects on plant growth, including enhanced leaf color, leaf length, and thickness. Additionally, PGJ demonstrates antibacterial properties, particularly against *E. coli*, and contributes positively to soil health by reducing pH and EC, which can help mitigate the soil reconditioning process. The catalytic enzymes in PGJ seem to be crucial for improving soil quality and facilitating nutrient absorption in plants. These findings underscore the potential of PGJ as both a plant growth enhancer and an eco-friendly soil amendment.

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