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# Assessment of Microbes in Liquid Tea Compost: A Qualitative Screening

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**ABSTRACT**

*An attempt to screening the microbes in the liquid tea compost. The liquid tea compost prepared from the waste tea leaves under shed drying. The water added to the compost, followed by brewing, aerating and sieving. The microbes, identified by Gram's staining, motility, biochemical test and selective media culture. In results, the shape of microorganisms viz. rods with bulged heads as positive, fluffy rods as negative, coccobacilli as negative and big rod as positively identified by Gram's staining. For motility, yeast was higher, moderate Clostridium sp. and Azotobacter sp. and slightly motile Pseudomonas aeruginosa. In biochemical tests, methyl red, VP and indole showed negative. For starch hydrolysis and $H_2S$ utilization, Azotobacter sp. was only positive while rests were negative. Azotobacter sp. and Pseudomonas aeruginosa positive while Clostridium sp. and yeast negative for catalase. Pseudomonas aeruginosa was negative while rests were positive for glucose fermentation. During sucrose fermentation, Clostridium sp. and Azotobacter sp. were positive but Pseudomonas aeruginosa and yeast were negative. Lactose fermentation and gelatin hydrolysis, all species were positive except yeast and Azotobacter sp. and Pseudomonas aeruginosa were positive while Clostridium sp. and yeast were negative for mannitol fermentation. The Azotobacter sp. was positive while others were negative for urease and Azotobacter sp. and Pseudomonas aeruginosa were positive while Clostridium sp. and yeast were negative in citrate utilization. In conclusion, the screening of microbes in a liquid tea compost, were identified and confirmed by the above-mentioned tests. The field study is suggesting to know the efficacy of this bio-fertilizer.*

***Key words:*** *Solid and liquid tea compost; Biochemistry and motility; Gram's positive and negative; Bio-fertilizer; Green compost*

## INTRODUCTION

It is well-known that bio-compost or bio-fertilizer replaces the synthetic fertilizer in agroecosystem and suitable for a green environment. There are several bio-composts have been investigated for soil fertility and growth enhancement of crop plant[1-2]. Besides these, liquid tea compost has also been prioritized in recent era[3-4].

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In general, composts harbour different types of beneficial microorganisms such as bacteria and fungi[5], which support the growth of plants, crops etc. Likewise, liquid tea compost also supports to inhabit several important bacteria and fungi and maintains the soil fertility and also helps in the growth of plants[6-8]. Presently, researchers concerned on aerated liquid tea compost to play a role of bio-pesticides[4,7,9-12] instead of non-aerated liquid tea compost, which means without fermentation[13-14]. According to Hilton[15], fermentation process leads to the cultivation of microorganisms in the compost.

Several researches have been documented the process of aerated as well as non-aerated compost preparation[4,7] but studies are lacking to detect microorganisms (bacteria and fungi) in the aerated liquid tea compost through the identification of microorganisms by Gram's staining, motility and biochemical test together for the confirming the qualitative assessment.

In the present study, it was an aiming to identify microorganisms with special reference to bacteria and fungi in the liquid aerated tea compost confirming by using several microscopic and biochemical techniques.

## MATERIALS AND METHODS

Shaban et al.[4] have mentioned the importance of compost tea with aeration and without aeration as well as beneficial for plants and soil structure by the presence of various microorganisms. The liquid compost was prepared from the tea compost by following the different steps as per methods developed by Kim et al.,[8]. The steps are exhibited in Fig 1 A – D.

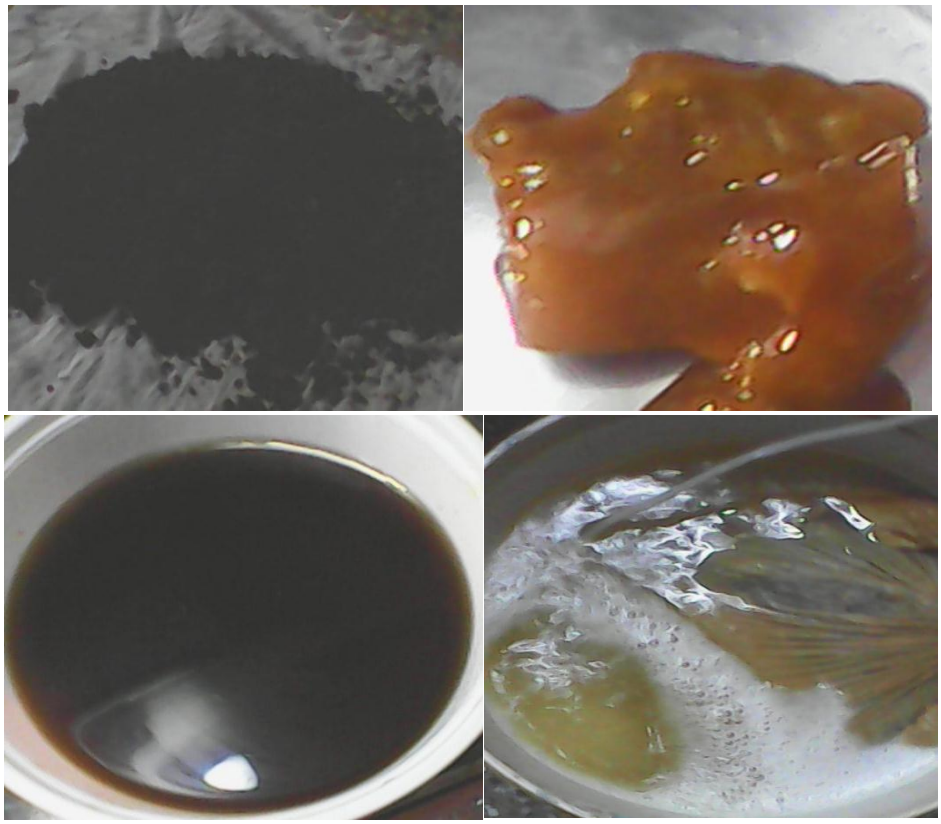

**Fig. 1: Different steps of liquid compost preparation from tea compost (A = drying of tea compost; B = molasses or jaggery prior to preparation; C = prepared molasses in liquid form and D = aeration of liquid compost from tea)**

**Preparation of tea compost**

The waste (leftover after making beverage) tea leaves were collected from different sources *viz.* tea beverage making shop and household kitchens. All the wet leaves about 5kg were kept in a bucket and allowed to get converted into compost. The bucket was covered to allow the decomposition process faster. In order to get quality compost, the compost was then dried in the open air. Till two weeks was observed visually and obtained compost after about 2 weeks by the method with some modifications developed by Kim et al.,[8].

**Preparation of liquid tea compost**

About 2kg of compost was taken with a bucket of 5gallon capacity. The water was then filled up to the rim of the bucket. Finally, to carry out the process of brewing, molasses (150gm/500ml) were added in the bucket.

**Preparation of molasses**

It has already been established that molasses are an important source of carbohydrate for bacteria. As it is the food for bacteria, its addition in the liquid tea compost, helped the luxurious growth of bacteria. The preparation of molasses was done by dissolving 150gm of sugarcane jaggery in the autoclaved water. The dark liquid is then filled in a 500ml bottle and mixed well with vigorous shaking to dissolve the remaining fine particles of jaggery. This liquid form of molasses was added to the liquid tea compost.

**Aeration of liquid tea compost**

The process of aerating in the liquid tea compost was done during the brewing process. Aeration was done by bubbling the liquid with the help of an aeration pump. Preferably, 3 pumps with bubblers were used in the process. Bubbling was carried out for 24hr – 48hr. Occasional stirring was also done to dissolve a higher quantity of oxygen in the liquid. This aeration is very important as it allows luxurious growth of aerobic microbes.

**Sieving of liquid tea compost**

After bubbling the dark liquid for about 48hr, the liquid was sieved by passing through a piece of autoclaved cloth. This was done to filter off the clear liquid as the compost particles remain behind in the cloth. By this process, a clear golden yellow coloured liquid is obtained, which was again subjected to aeration by bubbling for 24hr.

With all above-mentioned steps the liquid compost was prepared and this was used in various research works. Herein, the study of different microbial population was carried out.

**Study of microbes in liquid tea compost**

After the preparation of liquid compost from tea, a small quantity of sample was taken and processed in the laboratory, to detect the presence of beneficial microbes in liquid compost.

**Sample collection**

Some amount of solid tea compost was taken as a sample before mixing the compost with autoclaved water and setting up the brewing process. The solid compost was kept in a zip locked plastic packet and taken to the laboratory for microbiological test. The liquid compost was then collected in a sterile container. The filled-up sterile container was then wrapped in foil and taken to the laboratory for further biochemical analysis to confirm the particular strain of bacteria present in liquid compost.

**Isolation of bacteria and fungi from liquid tea compost**

Nutrient agar was prepared by using 0.5gm peptone, 0.3gm beef extract, 0.5gm NaCl, 1.8gm Agar, 100ml distilled water and potato dextrose agar (20gm potato, 2gm dextrose ,1.8gm agar, 100ml distilled water). For PDA (potato dextrose agar) medium, 8gm of smashed potato was dissolved in 100ml distilled water. All the media and reagents were autoclaved prior to culture. After autoclaving, very small amount (0.2gm) of solid compost was placed on the media with the help of sterile forceps. All the labelled plates were incubated at 37$^{o}$C.

**Serial dilution and spread plating on nutrient agar medium**

Serial dilution was done for the liquid tea compost. 9ml distilled water was taken in 9 labelled test tubes, marked as $10^{-1}$ to $10^{-9}$. The liquid sample (1ml) was then added to the first test tube. The tube was mixed thoroughly over the vortex for a few seconds and then 1ml

from the first test tube was added to the second tube and so on as per labelling. The labelled as $10^{-3}$, $10^{-6}$ and $10^{-9}$ were spread onto the media. 0.1ml from each test tube was taken with the help of sterile pipette and spread with the help of a sterile spreader. The plates were then incubated at 37$^{o}$C for overnight.

**Motility test for movement analysis**

The motility test was performed for liquid compost sample. As for the procedure, a drop of the liquid compost sample was taken on a clean slide, placed the coverslip with the help of a needle and observed under microscope, for the movement of bacteria at different rates[15].

**Gram's staining for bacteria identification**

As per the standard procedure[16], a drop of distilled water was taken on a clean slide. A loopful of inoculum was taken and a smear was made on the slide. This smear was air dried and the slide was passed through flame in order to heat-fix the smear. After obtaining a thin smear on the slide, Gram's staining was done. As per the procedure, firstly a drop of crystal violet was added, kept for 1min and then the slide was washed in running tap water. After that, a drop of Gram's iodine was added onto it, kept for 1min and then washed in alcohol. Next, safranin was added, kept for a min. and then washed in tap water. The stained slide was air dried, followed by drying with tissue paper in order to remove the excess moisture. The dried slide was observed under microscope with 100X oil immersion.

**Confirmatory biochemical tests for the identification of each species of microbe**

The citrate utilization test was carried out by using bacterial inoculum in separate test tube by the method developed by Beishir[17] with some modification. For starch hydrolysis test, same bacterial inoculum was used, as the test confirms the presence of a particular group of bacteria, hence those bacteria were positive for the test. The test procedure was followed by the method of Lanyi[18]. The hydrogen sulphide utilization test has been developed by Clarke[19] and the experiment was carried out as per the method of utilization of $H_2S$ by the test organism. In case of carbohydrate fermentation test, the experiment was carried out by the utilization of glucose, sucrose, lactose or mannitol by the test organism by the method of Reiner[20]. The tubes were observed for colour change and gas formation in Durham's tube. The urease test protocol was followed from James and Natalie[21]. The colour change of the media was recorded for particular bacteria. The gelatin liquefaction test is used to determine the ability of an organism to produce extracellular proteolytic enzyme, gelatinase that hydrolyze gelatin. This protocol was followed, developed a method by Thirstm[22]. The tubes were observed for solidification. The indole production test was carried out by the method of MacFaddin[23]. The colour change of the tube was recorded for particular bacteria. The methyl red test was carried out by the methods of Clarke and Kirner[24] and Cowan[25]. The colour change of the tube was recorded for particular bacteria. The VP (Voges-Proskauer) test was carried out by the method of MacFaddin[26]. The colour change of the tube was recorded for particular bacteria. The catalase test was carried out by the method of Beers and Sizer[27]. The bubbles seen on the drop marks in the tubes were recorded for particular bacteria.

**Selective media preparations and isolations of each microbe**

After the biochemical tests, identified the different samples of bacteria and fungi, the bacterial and fungal samples were then incubated on selective media, which was confirmed the presence of a particular species of bacteria and fungi. The sample was grown in CCFA (Cycloserine-cefoxitin fructose agar) and CFA (Catabolite repression of the colonization factor antigen) media prepared from an egg yolk–fructose agar base, the selective media for *Clostridium*. The medium was prepared as per the composition developed by George et al.,[28]. The selective and differential medium for isolation of *Clostridium difficile*. The sample was incubated in Ashby's medium and mannitol agar medium by the method of Rao[29] and Aneja[30] for the isolation of *Azotobacter* sp. The sample was processed in cetrimide agar

medium. The medium was prepared as per the composition developed by Lilly and Lowbur[31]. The selective medium was for isolation of *P. aeruginosa*. The sample was grown in YPD (Yeast Peptone Dextrose Agar) agar medium. The medium was prepared as per the composition developed by Ausubel et al.,[32]. The selective medium was used for isolation of yeast.

## RESULTS AND DISCUSSION

The study of microbes with special reference qualitative assessment of beneficial fungi and bacteria found in the liquid and solid compost prepared from tea. The isolation of bacteria and fungi from liquid tea compost, were identified based on Gram's staining, motility test, confirmatory biochemical tests and selective media culture to check their growth. It is well-known that tea compost contains many organisms, but is dominated by microbes, especially bacteria and fungi, which participated in the decomposition process [33-34]. The enhancement of microorganisms in the compost tea as described bio-fertilizer and may be suitable replacement of synthetic fertilizer[7], which is supporting the present results for obtaining bacteria and fungi in tea compost. In the present study molasses was used as substrate before fermentation, for the nutrient of microorganisms in tea compost, the common process described for the luxuriant growth of beneficial microorganisms[7,35]. The tea compost helps to control different pests[14,36].

The data of available bacteria and fungi were tabulated as per Gram's staining and microscopic observation (Table 1). The structure was visualized of each microorganism such as rods shaped with bulged heads (racket-like appearance), fluffy rods shaped, coccobacilli shaped and big rod shaped (Fig 2 A, B, C and D). As per Gram's staining, it was noted that the first structure was seen in Gram positive, second structure was found in Gram negative, third structure was observed in Gram negative and fourth structure was seen in Gram positive. As per the shape and staining as Gram positive or negative, all the strains were cultured in the selected medium to detect growth of particular bacteria or fungi inhabited in the liquid and solid tea compost. It was also found species specific growth in the selective medium such as *Clostridium* sp. were obtained in CCFA and CFA media, *Azotobacter* sp. were obtained in Ashby's medium and mannitol agar medium, *Pseudomonas aeruginosa* were observed in cetrimide agar medium and yeast was observed in selective media like yeast extract agar (Table 1). The characterization of microorganisms, Gram's staining is a suitable process. Madigan et al.,[37] and Naidu et al.,[7] have studied and identified bacteria and fungi in the tea compost when they used selective media for the study of growth of microorganisms and stained with Gram's stain. The confirmation made after visualizing under microscope that *Pseudomonas* sp., yeast, etc. found in the tea compost[7]. Chandra et al.[38] have documented that compost contained several types of bacteria of which 84.8% isolated Gram-positive in which 85.7% were rods and 14.3% cocci, while the rest 15.2% were Gram-negative, observed as rod shaped. The present study with tea compost has similarities with previous researches[7,38].

**Table 1: Different shapes of microorganisms present in liquid tea compost**

| Sl. No. | Description | Gram's staining | Microorganisms |
|---|---|---|---|
| 1. | Rod shaped with bulged head (racket-like appearance) | Gram positive | *Clostridium* sp. |
| 2. | Fluffy rod shaped | Gram negative | *Azotobacter* sp. |
| 3. | Coccobacilli shaped | Gram negative | *Pseudomonas aeruginosa* |
| 4. | Big rod shaped | Gram positive | Yeast |

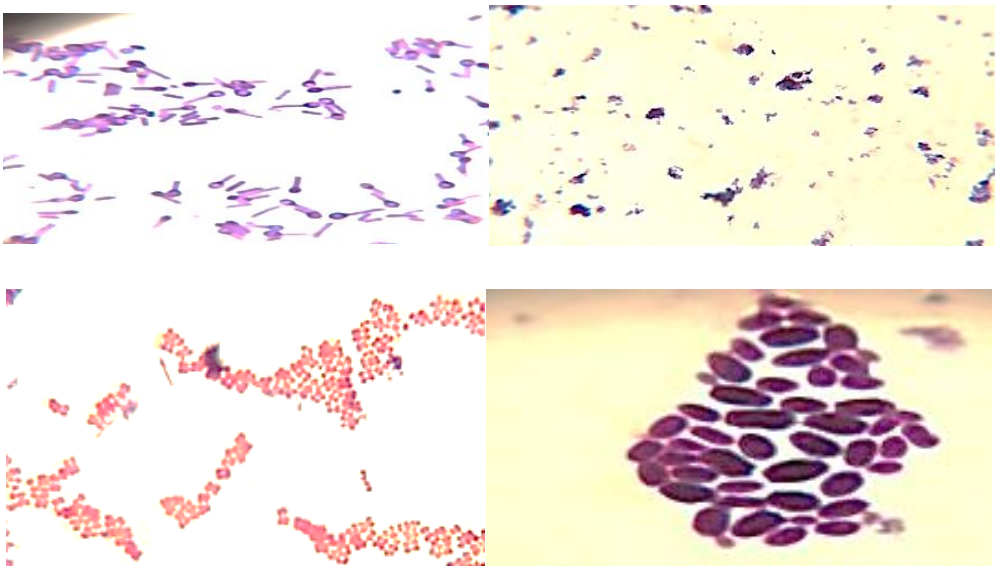

**Fig. 2: Photomicrograph of different shapes of microorganisms found in liquid tea compost (A = Racket-like appearance; B = Fluffy rods shaped; C = Coccobacilli shaped and D = Big rods shaped)**

The performance of motility for specific bacteria and fungi were studied under microscope and tabulated in Table 2. It was observed that yeast showed high motility, *Clostridium* sp. and *Azotobacter* sp. were moderately motile and *Pseudomonas aeruginosa* slightly motile. Interestingly, the motility of microorganisms served an important role to identify specific bacteria and/or fungi in the particular medium[39]. It was known that flagella bearing microbes are motile, few bacilli are known to be motile and cocci are non-motile[39-40], the present results of motility with an agreement of another study.

**Table 2: Motility test for specific bacteria and fungi isolated from liquid tea compost**

| Sl. No. | Motility types | Microorganisms |
|---|---|---|
| 1. | Moderate | *Clostridium* sp. |
| 2. | Moderate | *Azotobacter* sp. |
| 3. | Low | *Pseudomonas aeruginosa* |
| 4. | High | Yeast |

The confirmatory biochemical tests (Table 3) revealed that all bacteria and fungi showed negative results for methyl red test, VP test and indole test (Fig 3A, B and C). In case of starch hydrolysis test and $H_2S$ utilization test, it was found positive data was observed only for *Azotobacter* sp. while the rest showed negative data for other bacteria and fungi (Fig 3D and F). The bacteria, *Azotobacter* sp. and *Pseudomonas aeruginosa* showed positive data while *Clostridium* sp. and yeast showed negative data for catalase test (Fig 3E). In case of glucose fermentation test, *Pseudomonas aeruginos*a showed negative data while positive data were found for *Clostridium* sp., *Azotobacter* sp. and yeast (Fig 3G), in the sucrose fermentation test, *Clostridium* sp. and *Azotobacter* sp. showed positive data and negative data were observed in *Pseudomonas aeruginos*a and yeast (Fig 3H). In the lactose fermentation test and gelatin hydrolysis test, all three species of bacteria showed positive data except yeast (Fig 3I and K) and in the mannitol fermentation test, *Azotobacter* sp. and *Pseudomonas aeruginosa* showed positive data while *Clostridium* sp. and yeast showed negative data (Fig J). The *Azotobacter* sp. was only showed positive data while other bacteria

and fungi showed negative data for urease test (Fig 3L). In case of citrate utilization test, *Azotobacter* sp. and *Pseudomonas aeruginosa* showed positive data while *Clostridium* sp. and yeast showed negative data (Fig 3M). From the past decade, many researchers have developed methods in bacteriology to detect the enzyme reactions by bacteria and fungi[41-46]. It has been well-established to know the presence and/or absence of specific microorganism in the culture medium and the positive or negative results indicated the chemical reaction within the cell of microorganisms by fermentation, reduction of nitrate and of methylene blue, production of indole, hydrogen sulphide and acetoin, and hydrolysis of gelatin, starch and urea[47]. In the present study, the positive and negative results confirmed the presence and absence of bacterial and fungal strain with an evidence of earlier research.

**Table 3: Biochemical tests for specific bacteria and fungi isolated from tea compost**

| Sl. No. | Biochemical parameters | Bacteria and fungi species | | | |
|---|---|---|---|---|---|
| | | ***Clostridium* sp.** | ***Azotobacter* sp.** | ***Pseudomonas aeruginosa*** | **Yeast** |
| 1. | Methyl red test | - | - | - | - |
| 2. | VP test | - | - | - | - |
| 3. | Starch hydrolysis test | - | + | - | - |
| 4. | Catalase test | - | + | + | - |
| 5. | $H_2S$ utilization | - | + | - | - |
| 6. | Indole test | - | - | - | - |
| 7. | Glucose fermentation | + | + | - | + |
| 8. | Sucrose fermentation | + | + | - | - |
| 9. | Lactose fermentation | + | + | + | - |
| 10. | Mannitol fermentation | - | + | + | - |
| 11. | Urease test | - | + | - | - |
| 12. | Citrate utilization | - | + | + | - |
| 13. | Gelatin hydrolysis | + | + | + | - |

**+ = Positive and - = Negative**

**Fig. 3: Biochemical identification for presence or absence of microorganisms in liquid compost tea (A: Methyl red; B: VP; C: Indole; D: Starch hydrolysis; E: Catalase; F: $H_2S$ utilization; G: Glucose fermentation; H: Lactose fermentation; I: Sucrose fermentation; J: Manitol fermentation; K: Gelatine hydrolysis; L: Urease; M: Citrate utilization)**

## CONCLUSION

In conclusion, the qualitative assessment of microorganisms in a liquid tea compost revealed that beneficial fungi and bacteria found in the liquid and solid compost prepared from tea. The isolation of bacteria and fungi from liquid and solid tea composts, were identified and confirmed by on Gram's staining, motility test, selective media culture to check their growth and biochemical tests for their presence and absence in the tea compost. Herein, the enhancement of microorganisms in the compost tea as described bio-fertilizer and may be suitable replacement of synthetic fertilizer[7], which is supporting the present results for obtaining bacteria and fungi (*Clostridium* sp., *Azotobacter* sp., *Pseudomonas aeruginosa* and yeast) in tea compost. The present work is suggesting in the future to detect the role of tea compost as bio-fertilizer by using in the field and looking the efficacy in the growth of agricultural crops.

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**Conflict of interest**

The authors declare none of conflict of interest in the present work.

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