Investigation of microorganisms associated with the spoilage onions

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Abstract— The purpose of the study was to look into the microbes responsible for the deterioration of onion bulbs near Vadodara. Sample was obtained from a neighbourhood market. Analyses were conducted using culture characterisation, Gram staining, and biochemical test. Staphylococcus sp, Bacillus sp, Pseudomonas sp, Klebsiella, and Escherichia coli were the bacteria species that were isolated, whereas Aspergillus sp, Mucor were the fungi. New, appropriate pre-harvest and post-harvest disinfection techniques must be developed. From this study conclusion is that spoiled onion bulbs are a source of both bacterial and fungal organisms that are known to induce spoiling. It may also be the result of the onions being exposed to unfavourable conditions during transportation, storage, and inadequate packaging, which could raise the temperature and humidity of the product. Several of the isolated organisms were amoxicillin-resistant yet susceptible to streptomycin, chloramphenicol, and other antibiotics.

Keywords: deterioration, fungi, microbes, onion bulbs

I. INTRODUCTION

The onion, or *Allium cepa*, is a significant vegetable crop that is grown all over the world for both human consumption and its economic worth to farmers. It is a member of the *Alliaceae* family, and its bulbs, that areconsumed every day in every household for seasoning and flavouring, are the reason it is cultivated. Because to the sugars, vitamins, and minerals found in onions, they are an important dietary component. Onions are available in three distinct varieties: Red, White, and Oriental. The majority of onion varieties reportedly include89% water, 4% sugar, 1% protein, 2% fibre, and 0.1% fat [1]. *Allium cepa L*., the onion (from the Latin cepa, "onion"), is the most commonly farmed among the genus *Allium.* Other names for it include bulb onion and regular onion. In reality, onions are biannual or perennial plants, even though they are usually treated as yearly and collected in the initial planting season. The bulb that grows at the bottom of the onion plant begins developing and produces an arrangement of hollow, bluish-green leaves when a certain amount of daylight is reached. The bulbs are composed of compressed and elongated subterranean stems and mushy modified scales, or leaves that surround a centralized bud at the apex of the stem. [2]. Garlic and onions, or Allium sativum and *Allium cepa L*., are two amongst the first domesticated plants and are used for culinary and medicinal purposes. In reality of course, these commonly consumedcrops are an abundant supplier of numerous of Plant based nutrients that are recognised like an essential components of the Mediterranean diet, in addition to being utilized in the handling and therapy of several illnesses, including tumors, heart illness, excess weight, high cholesterol levels, Type 2 diabetes, high blood pressure, eye conditions, and gastrointestinal disorders (such as indigestion, flatulent colic, and abdominal pain pain) [3].onion farmers nearlyalways preserve their crop to maintain a steady supply during the months while fresh product is scarce. Onionloss during storage is frequently brought on by bulb rots. In particular in tropical and subtropical environments,moulds and fungi are significant diseases of fruits and vegetables [4]. Reduced quantity and quality of onionsaffect market value, which is why storage rots are important [5]. Mycotoxin contamination of the impacted material is another significant consequence that is frequently disregarded [6].

Many heat, non-heat, chemical in nature, and biologic remedies have been researched in an effort to deactivate the bacteria associated with onion products. With numerous different combinational therapies, the plasma therapy has been widely used. *Bacillus cereus*, *Staphylococcus*, and *pseudomonas cepacian* are among the pathogens that are screened for in onion items. Onion items have been reported to have a variety of spoilage fungi, which include *fusarium oxysporum ,Rhizopus oryzae, Penicillium sp., Aspergillus, Botrytis sp*, as well as *Alternaria sp* [7].One of the most popular vegetable harvests on the world market is the onion. Since onions are a root crop, theybecome afflicted with a number of soil-borne illnesses as they develop. Although these illnesses may not showsymptoms in the crops while they are in the field, pathogenic bacterial and fungal contaminations persist evenafter harvest and can cause havoc, leading to the full spoiling of the bulbs when stored in a healthy environment[8]. The most frequent pests and diseases that might damage the crop include the *Delia antiqua*, Stem and bulb nematodes, and various rot-causing fungus. [2]. In order to meet demand during the off-season, 30-40' o of the produce from the rahi season onion is stored, of which 15-20% is lost owing to damage brought on by storage illnesses. The post- harvest diseases that affect onions the most include brown rot, neck rot, blue mould, and black mould. Black mould and blue mould are the most common types of soft rot [9]. Numerous earlier and after harvesting factors, including as genetic qualities, soil characteristics, environmental variables, and administration strategies, can be linked to the presence of pathogenic bacteria in onion bulbs and the ensuing decay of onion bulbs. The grey mould *(Botrytis spp.)*, black mould (*Aspergillus spp*.), blue mould (*Penicillium spp*.),

bacterial rot (*Erwinia spp*.), and pseudomonas spp. are significant onion pathogens [10]. The contamination of the onions is caused by airborne spores of *Aspergillus sp. and Staphylococcus sp.* During handling, processing, and storage, *yeast, mould, and Pseudomonas sp*. are present. Ringworm and aspergillosisare brought on by Aspergillus sp. Post-harvest, *Penicillium allii* causes blue mould rot. Humans can get food poisoning from bacteria such *E. coli, Staphylococcus, Bacillus, and Pseudomonas* [11]. Onions are connectedto microorganisms that might lead to rotting. Typically, this spoiling happens throughout the harvest, post- harvest, shipping, marketing, and storage processes. In tropical nations, storage is done at room temperature (between 24 and 320 degrees Celsius) and varying relative humidity levels based on the time of year. The quality of the onion bulbs, the environmental factors, the handling and processing circumstances, the storage facility conditions, and the onions themselves are all factors in the presence of fungi in onion bulbs. Both humans and animals have been known to contract diseases from these fungi [12].

II. MATERIAL AND METHOD

Material: Nutrient broth, Nutrient agar , MacCkonkey agar ,Eosin methycylene blue , Petridishes ,Testtubes,Conical Flask , Autoclave , Beakers , Distilled water ,Universal bottles, Alcohol , Mueller Hinton Agar**,** Sabouraud agar, Antibiotic disc - Streptomycin, chloramphenicol and amoxicillin , Other - Flame, forcep andscale, mortar and pestle ,Gram stains (Crystal violet, Gram's iodine solution, safranin)

The analysis required that the onion bulbs be randomly selected from nearby markets, packaged in uncontaminated plastic bags, and transported at the laboratory.

II.I. CULTURING AND ISOLATION OF ORGANISMS

The decayed onion bulb's outer layer was cleaned with 70% alcohol. For each sample, using a sterile cutting blade, a single gram of the spoilt onion vegetable was sliced and placed individually into a sterile motor and pestle. After being crushed with sterile distilled water, each sample's single milliliters of sample was transferred to 9milliliters of the clean nutrient brothin a universal bottle, where it was left for 15 minutes. Each sample from 101 to 105 was serially diluted using1 ml from each test tube.

Through the use of sterile storage, each media was placed onto a germ-free Petri plate, and each media was then supplemented with a bacterial sample obtained from the sample's serial dilution. After thereafter, setup was permitted. The procedure was carried out once more for the other sample. After that, the inoculation plates were turned upside down and kept at 37°C for a full day. To stop bacteria from growing in the media used to grow fungi, an antibiotic called chloramphenicol was added before it cooled and solidified. The plates were then incubated for 5 to 7 days at room temperature.

For each plate, a bacterial and fungal count was done to figure out how many bacteria and fungi were growingthere. This was calculated by counting the entire plate and multiplying the result via the factor of dilution [9, 10].

II.II. MACROSCOPIC ANALYSIS AND COLONY RECOGNITION

The colonies in the plate were first morphologically examined under a microscope for colonial appearance, size, elevation, form, edge, consistency, colour, opacity, hemolysis, and pigmentation in order to characteriseand identify the colony isolates. The results were then documented. The isolates were identified and defined using the gram response, biochemical processes response, and characteristics of culture.

II.III. IDENTIFYING FUNGUS AND BACTERIA USING THE GRAM REACTION

Wet mount technique microscopy was used to view fungi isolates. A loop of the isolated colony was taken out and placed on a grease-free slide. Lactophenol cotton blue was then applied, the slide's surface was coated with cover glass, and the fungus was examined under a microscope. It was observed using a 40x objective lens microscope. On a plate,fungus colonies were examined under a microscope and for colour and appearance [1].

To distinguish between gram positive and gram negative species, this was done. A tiny bit of microbial development was taken out of the culture and placed with a small amount of water on a sterile surface .A thin smear of the speck was created on the slide by emulsifying it. After then, the smear was heated by passing it over a flame. Following a 60-second crystal violet infusion, it was cleaned with water from distillation, iodine was applied for half minute, it was again cleaned with water from distillation, and finally alcohol was used to remove any remaining colonisation. Finally, a 60-second safranin flood was applied to the smear to counterstain. Following a distilled water wash,it will be left to air dry. At an objective lens of 100x, oil immersion was utilised. In contrast to red, purple colouring give gram-positive bacteria.

II.IV. CHARACTERIZATION OF BACTERIA (BIOCHEMICAL TESTS) [13]

II.IV.I. CATALASE TEST: This was used to differentiate between the microorganisms that produce the enzyme catalase. The catalase test was conducted according to protocol. A tiny portion from the bacterial growth was transferred to a clean glass slide using a sterile inoculating loop and a drop of H_2O_2. The presence of catalysts, as demonstrated by bubbling, signified a positive test result for bacteria, but the absence of a bubble demonstrated a negative test result.

II.IV.II. INDOLE TEST: The purpose of this experiment was to determine whether the test organism generated indole, an essential enterobacteria indicator.procedures were followed as directed by After adding 1% tryptophan to 5 milliliters of sterile peptone water, each isolate was added. The combination was then incubated at 37°C for a whole day. The culture was given 0.5 ml of Kovac's reagent, which was added and carefully mixed. Red indicated a positive result for microorganisms, whereas yellow indicated a negative result.

II.IV.III. CITRATE UTILIZATION TEST: This test assesses the ability of an organism to use citrate as a carbon source. A tiny quantity of Koser's citrate medium was added to each isolate, and it was then cultured for 72 hours at 37°C. A positive citrate test is indicated by the appearance of a vivid blue tint, despite the medium's original green color indicating a negative test.

II.IV.IV. METHYL RED: Enterobacteria were identified by MR testing based on their ability to produce and maintain stable glucose end products. A little quantity of each isolate was added to the glucose phosphate peptone water medium, which was subsequently cultured for 48 hours at 37°C. A few drops of methyl red were added to the culture. The color red was used to denote MR positivity.

II.IV.V. VP TEST: A pure culture of the organism to be tested should be added to the VP broth tube for inoculation.A 24-hour incubation period at 35°C.After adding 0.6 mL of 5% alpha naphthol, add 0.2 mL of 40% Potassium hydroxide. To expose the medium to ambient oxygen, give the tube a little shake and then let it rest undisturbed for 10 to 15 minutes. An indication of a positive VP test is a pink-red surface color that occurs 15 minutes or more after the reagents are added.

II.IV.VI. Oxidative test: Pick up a piece of filter paper that has been saturated with the substrate tetramethyl-p- phenylenediamine dihydrochloride. Apply sterile distilled water to the paper to moisten it. With a platinum or wooden loop, select the colony to be examined, and smear it onto the filter paper. During 10 to 30 seconds, the infected portion of the paper should turn a deep blue or purple colour. Test resultsare positive if deep purple colour develops within 10 seconds. Colorless means the test was negative.

II.V. ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antibacterial sensitivity test was employed to examine how susceptible various bacterial species were to various drugs and artificial substances. To determine whether bacteria were sensitive to the selected antibiotics, three methods were used: the zone size interpretation chart, the traditional disc diffusion method, and antibiotic susceptibility testing. Commercially available paper discs were impregnated with several antibiotics, including chloramphenicol, ampicilin and streptomycin, which will be tested against the isolates. Bacterial isolate inoculums were eachcultured in separate plates in Mueller Hilton broth at 37°C.Following an incubation time of 24 hours at 37°C, the zones of inhibition on the plates were measured and examined [1].

III. RESULT AND DISCUSSION

The bacteria that were identified in the current investigation were *Staphylococcus sp., Bacillus sp., Pseudomonas sp., Escherichia coli, Klebsiella, mucor*. After taking small amount of spoiled onion, it was crushed and then serial dilution was done. So 1-2 drops of serial dilution was added on different agar media like nutrient agar, MacCkonkey agar and Eosin methycylene blue agar and then kept for incubation .The bacterial colonies were there. For fungus grew on Sabouraud agar. Bacteriostatic

sabourand agar prevents the growth of bacteria since it contains antibiotics. Contrarily, nutrient agar contains an antimycotic substance that prevents the growth of fungus.

III.I. GRAM STAINING

By performing gram staining it can say that *E.coli, Pseudomonas and klebsiella* are present because of the gram-negative rods (figure 1, 2, 3). *Staphylococcus sp*.which is a Gram- positive cocci, and *Bacillus sp*., is a Gram-positive rod (figure4, 5). *Mucor and A.niger* were also found with the help of lactophenol cotton blue (figure 6, 7). Gram-positive bacteria continue to stain dark violet or purple after being washed with pure alcohol and water. This indicates that the Gram stain continues to color the bacteria blue or purple. A decolorizing solution is used to rinse the bacteria after they have been stained with crystal violet in a Gram stain test. While Gram-positive bacteria retain their crystal violet color, Gram-negative bacteria become reddish-pink stained when a counterstain such as safranin or fuchsin is introduced after washing. This is primarily due to the differences in the construction of respective bacterial cell walls. The peptidoglycan layer of Gram-positive bacteria is thicker and retains the color of crystal violet, while the coating of Gram-negative bacteria is thinner. Gram-positive bacteria have a lot of peptididoglycan in their cell walls, which is crucial for the dye's retention.

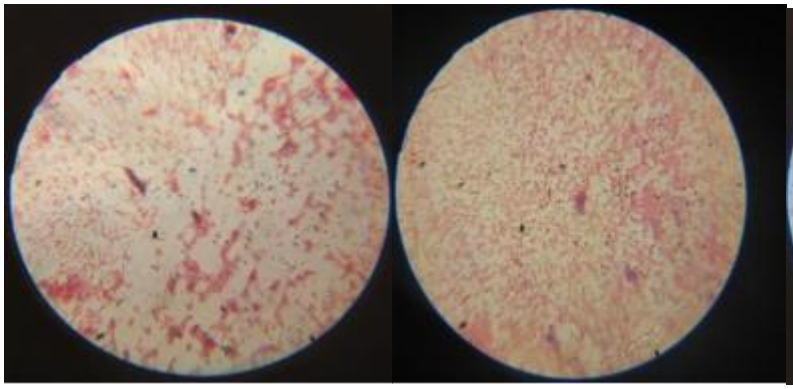

Figure 1. ***E.coli***

Figure 2. ***Pseudomonas***

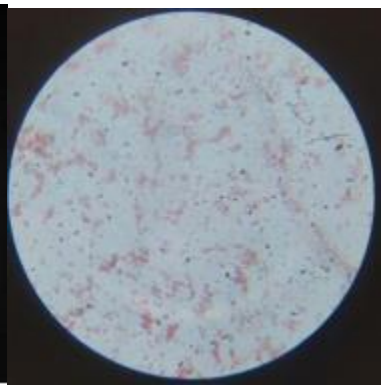

Figure3. ***Klebsiella***

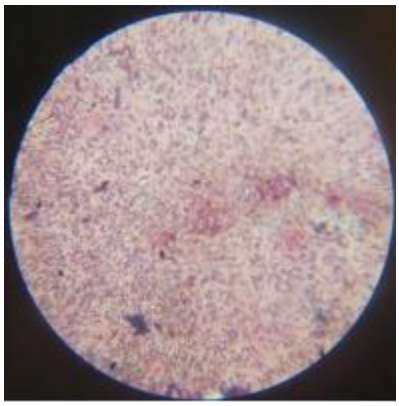

Figure 4. ***Staphylococcus***

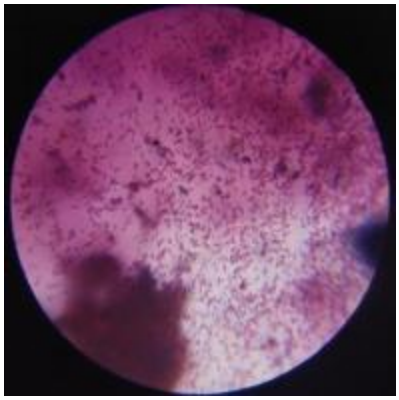

Figure 5. ***Bacillus***

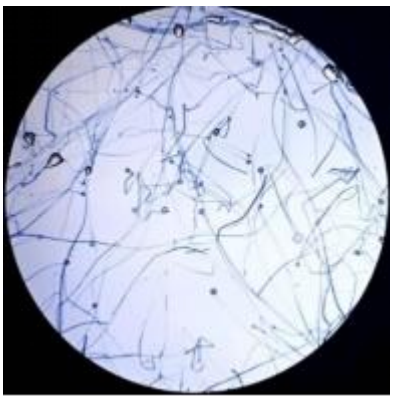

Figure 6. Mucor

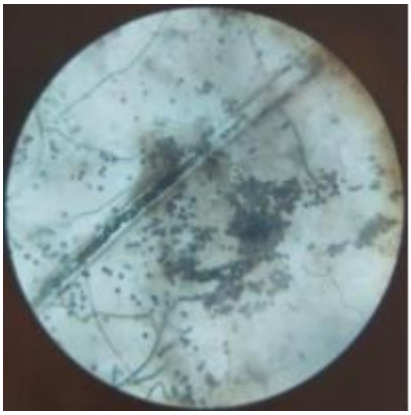

Figure 7. ***Aspergillus niger***

III.II. BIOCHEMICAL TEST AND COLONY FORMING UNIT

Biochemical test was performed like catalase test (Figure 8), oxidase test (Figure 9), MR test (Figure 10),VP test (Figure 11), citrate utilization test (Figure 12) and indole test (Figure 13) etc.

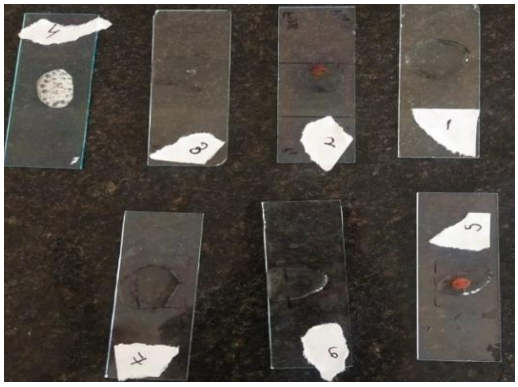

Figure 8. Catalase test

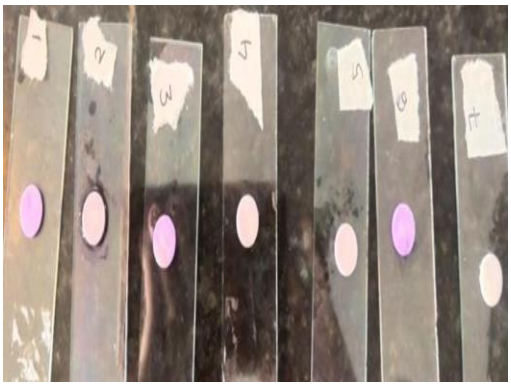

Figure 9. Oxidase test

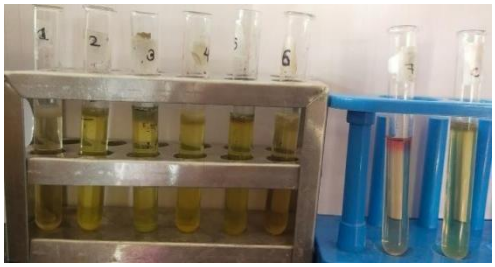

Figure 10. MR test

Figure 11. VP test

Figure 12. Citrate utilization

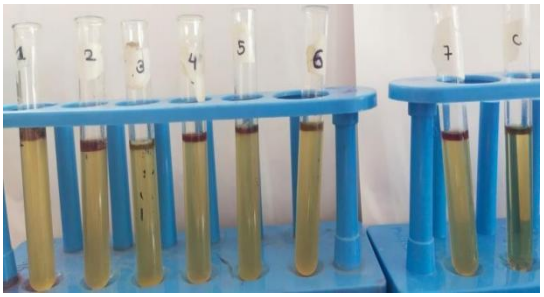

Figure 13. Indole test

According to data from Table 1, *Escherichia coli, Pseudomonas sp, Staphylococcus sp, Bacillus sp, andKlebsiella spp*. were detected in samples of spoiled onions obtained from local markets.*E.coli, Pseudomonas and klebsiella* are of gram-negative rods. *Staphylococcus sp*.which is a Gram-positive cocci, and Bacillus sp.,is a Gram-positive rod. In which *E.coli* gives negative result in catalase , oxidase,MR, citrate test and positive in vp and indole test.*Pseudomonas* is positive in oxidase, citrate and negative in catalase, MR, VP, and indole test.Staphylococcus is negative in oxidase and MR test whereaspositive in catalase, VP, citrate and indole. *Klebsiella* is negative in catalase , oxidase and indole whilepositive in MR,VP, Citrate. *Bacillus* is negative in catalase, MR, VR, indole while positive in oxidase and citrate.

Table 1. Biochemical test for isolated bacteria

Test	*E.coli*	*Pseudomonas*	*Staphylococcus*	*Klebsiella*	*Bacillus*
Catalase	-	-	+	-	-

Oxidase	-	+	-	-	+
MR	-	-	-	+	-
VP	+	-	+	+	-
Citrate	-	+	+	+	+
Indole	+	-	+	-	-
Gram Staining	-	-	+	-	+

The total colony count in Colony Forming Unit (cfu/g) of different bacteria and fungi produced from the rotten onion is displayed in Table 2 based on data acquired from local markets. The results show that 2X105cfu/ml was least and 7X105cfu/ml was the largest number of bacteria identified.

Table 2. Total colony count from samples collected from local market

No	Colony count
1	7X105cfu/ml
2	3X105cfu/ml
3	2X105cfu/ml.

III.III. CHARACTERIZATION AND IDENTIFICATION OF ISOLATED FUNGUS AND MORPHOLOGICAL CHARACTERISTICS OF BACTERIA FROM ONION

From the samples of spoiled onions, fungus such as Aspergillus sp. and Mucor were found and describedin Table 3. In a Sabourand Dextrose Agar plate, Mucor showed white coloration and thick, non-septatehyphae under a microscope. Black colonies of Aspergillus sp. were seen. Morphological characteristic of bacteria isolated is in table 4.

Table 3. Characterization and identification of the isolated fungus

Organism	Microscopic	Morphology
Mucor	Black sporongiospore, thick non-septate hyphae	entire plate covered in white growth
Aspergillus sp	Filamentous coupled with branching mycelia or dense hyphae with septate structures	Many Black colony all together

Table 4. Morphological characteristic of bacteria isolated

Bacteria	size	Shape	Gram's nature
E.coli	small	Rod shape	Gram negative
Pseudomonas	large	Rod shaped	Gram negative
Staphylococcus	Large	Circular, Grape like structure	Gram positive
Bacillus	Large	Rod shape	Gram positive
Klebsiella	Large	Rod shape	Gram negative

III.IV. ANTIBIOTIC ASSAY

As presented in table 5 in *E.coli* the zone of inhibition of streptomycin is 17mm, Chloramphenicol is 24mm and in Amoxicillin it is 5mm. While in *Bacillus* the zone of inhibition of streptomycin is 15mm,Chloramphenicol is 23mm and in Amoxicillin it is 2mm. In *Pseudomonas* the zone of inhibition of streptomycin is 23mm, Chloramphenicol is 9mm and in Amoxicillin it is 8mm whereas in *Klebsiella* the zone of inhibition of streptomycin is 19mm, Chloramphenicol is 9mm and in Amoxicillin it is 3mm.In *S.aureus* the zone of inhibition of streptomycin is 19mm, Chloramphenicol is 29mm and inAmoxicillin it is 7mm. So we can say that amoxicillin is not that effective against these bacterias. *E.coli*,*S.aureus and Bacillus* is more sensitive to chloramphenicol, while *Klebsiella and Pseudomonas* is moresensitive to streptomycin. The antibiotic sensitivity of bacteria isolates is displayed in table 5 data. Several of the isolated organisms were amoxicillin-resistant yet susceptible to streptomycin, chloramphenicol (figure 14, 15, 16, 17, 18). By performing antimicrobial activity so many medicines canbe made against these microbes.

Table 5. Antibiotics employed on bacterial isolates and their pattern of antibiotic sensitivity from the spoiled onions

Bacteria	Streptomycin	Chloramphenicol	Amoxicillin
E.coli	17mm	24mm	5mm
Bacillus sp.	15mm	23mm	2mm
Pseudomonas sp.	23mm	9mm	8mm
Klebsiella sp.	19mm	9mm	3mm

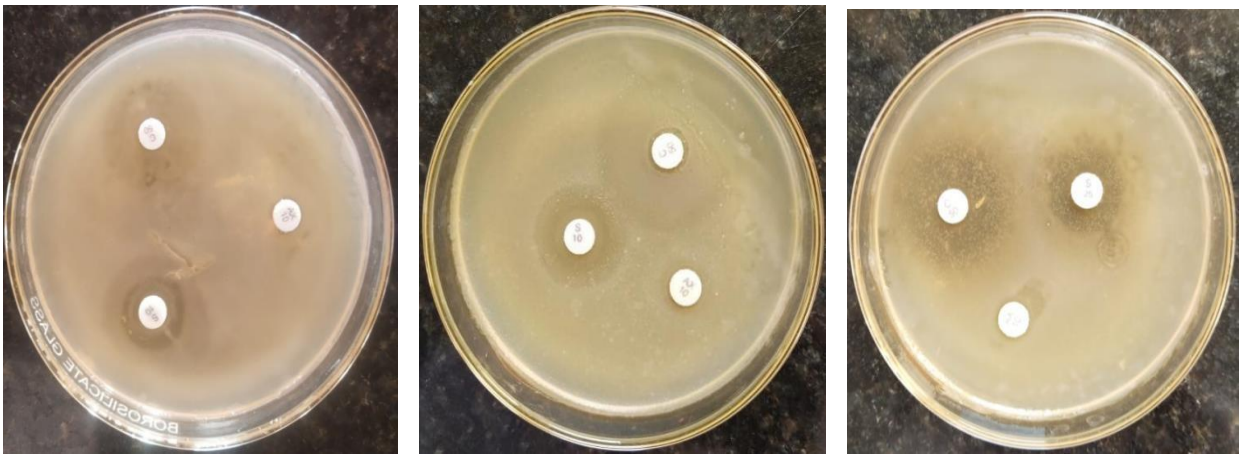

Figure 14. ***Bacillus***

Figure 15. ***Staphylococcus spp***

Figure 16.*E.coli*

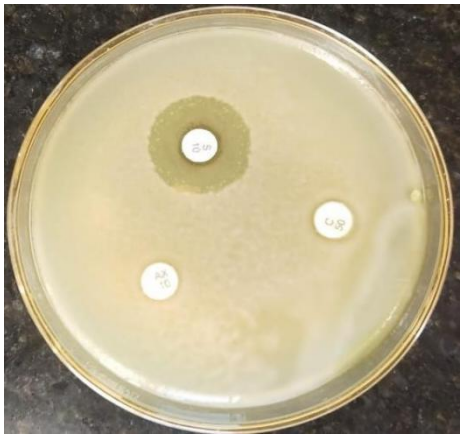

Figure 17. ***Pseudomonas spp***

Figure 18. ***Klebsiella spp***

The recent examination found the following organisms: *Aspergillus, mucor, Klebsiella, Bacillus, Pseudomonas,* and *Escherichia coli, Mucor*. *Staphylococcus sp., Bacillus sp., Pseudomonas sp*., *Escherichia coli,* and *Klebsiella* are the bacterial isolates while *Aspergillus sp.* and *Mucor* are the fungus isolates. Most of the bacteria that were collected flourished on Nutrientagar, whereas mold development was observed on Sabouraud agar.This is due to the fact that sabourand agar is bacteriostatic (contains antibiotics) which prevents the development of bacteria. Contrarily,nutritional agar contains an antimycotic substance that prevents the growthof fungus. It has been confirmed that all of the microbesthat were successfully isolated participate in fruit rot. *Aspergillus sp*. appears in onion bulbs with a highfrequency. These fungi may infect humans and animals with illnesses like ringworm and aspergillosis by spreading through the air, breeze, water, soil, and even the processor. These are the sources of dangerousmycotoxins with a high potency for human health.

Aspergillus niger, a common fungus found in nature, is said to be able to survive among crop yields of onions by growing as a soil saprophyte in or on preserved bulbs in the planting area. Then gus may infiltrate onion bulbs in the field or while they are being stored because it generates a range of enzymes and poisons when it finds damaged tissues. *Aspergillus niger* has been linked to onion seeds grown in hot climates. It infects onion seedlings through ground and naturally contaminated seeds, which causes 30–80% of the onion bulbs to rot.

Poor packing, transportation, and storage practises might be at blame for this contamination. To prevent spoiling due to the heat generated by the polythene bags, onions wrapped in nylon must be put out in the open after purchase. Avoid purchasing spoiled onions from the market. To avoid foodpoisoning, nevertheless, certain precautions should be used when preparing onions. Additional study on onion management practices and market controls for quality is required to avoid post-harvest losses and food contamination.

IV. CONCLUSION

From this study we can say that spoiled onion bulbs are a source of both bacterial and fungal organismsthat are known to induce spoiling. It may also be the result of the onions being exposed to unfavourableconditions during transportation, storage, and inadequate packaging, which could raise the temperatureand humidity of the product. Several isolated organisms were amoxicillin-resistant yet susceptible to streptomycin, chloramphenicol. By using the dichotomous key for identifying bacteria by gram staining and biochemical test, we can infer that the sample may contain *E. coli, Pseudomonas sp, Staphylococcussp, Klebsiella sp, Bacillus, mucor, and Aspergillus,* and that these bacteria can then be identified using gene sequencing.

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