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Identification and characterization of Aspergillus species of fruit rot fungi using microscopy, FT-IR, Raman and UV–Vis spectroscopy

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ABSTRACT

During the investigation of fungal isolation from fruit, the major genera were *Aspergillus*, *Penicillium*, *cladosporium*, *Alternaria*, *fusarium*, *Colletotrichum* were found. Among them *Aspergillus* (15 species) was found major dominant on different fruits. Fifteen different *Aspergillus* species *viz. Aspergillus brasiliensis*, *Aspergillus phoenicis*, *Aspergillus carbonarius*, four *Aspergillus flavus*, *Aspergillus acidus*, *two Aspergillus awamori*, *Aspergillus aculeatus*, *Aspergillus eucalypticola*, *Aspergillus oryzae* and two *Aspergillus* Spp. have been differentiate and identify using morphology (microscopic technique), Fourier Transforms Infrared spectroscopy (FTIR), Raman Spectroscopy (RS) and UV–visible spectrophotometry (UV–vis). The fungal mass in powder form was used in present study. In FTIR the finger print region is important for the characterization of Aspergillus because this region is unique and contains peaks indicating the presence of DNA. From the results were found Fourier transform infrared (FTIR) technique and Raman spectroscopy a useful tool, sensitive, fast, economical, accurate, not require sample preparation and successfully used to identify fungi.

1. Introduction

Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to maintain healthy life to human mankind [1]. However, despite all these tremendous benefits of fruits to human well-being, pathogenic attacks threatened the shelf-life of fruits and degrade their economic value. Fruits are mainly exposed to microbial contamination through contact with soil, dust and water and by poor handling or during postharvest processing. This makes them to harbor a wide range of microorganisms [2,3] especially fungi. India achieved a record horticultural production of 314.67 million metric tons in 2018–19 [4]. Storage is the most important cause of post-harvest losses for all types of food in India. Losses due to postharvest disease may occur at any time during postharvest handling, from harvest to consumption. When estimating postharvest disease losses, it is important to consider reductions in fruit quantity and quality, as some diseases may not render produce unsaleable yet still reduce product value. The fungi have importance in nature rotating organic plant materials, important in the environment, food production, pharmacy and industry. Fungi are almost the only organisms that can degrade cellulose and lignin, they are indispensable to ecosystems [5]. Rotting fruits are the main food sources of fungi, containing essential nutrients to grow fungi such as Aspergillus [6]. Aspergillus causes the rotting of different fruits such as oranges, apples, guava, grapes, water melons and papaya, mold grows in different colors, mostly black mold that grows on most fruits. Aspergillus adapts to the physical, chemical and biological environment changes that are rapidly growing and developing. Aspergillus has a variety of genes, many of which grow on the outer layer of damaged fruits. The genus Aspergillus is taxonomic group organisms, it has diverse properties in agriculture, pharmaceutical, industrial and biological characteristics make it cultivation important [7]. Aspergillus are shown in damaged fruits by their infected as decay and rot, they have different colors rot, and the most common is a black rot. Some genus of Aspergillus important for biotechnology applications such as *A. oryzae*. Large amounts of proteins have been produced from Aspergillus oryzae, so it was used in food fermentation in Japan [7,8]. Aspergillus able to produced biologically active chemical compounds such as antibiotics, mycotoxins. Aspergillus flavus is able to produce mycotoxins as cyclopiazonic acid and Aspergillic acid [9]. Aspergillus is using for the biosynthesis of nanoparticles because it has enough amount of enzymes and it cultured on different medium [10]. The aim of this study is to identify Aspergillus species, which collected from infected fruits from different markets in Aurangabad, India by using microscopic technique, FTIR spectroscopy, Raman spectroscopy and UV–Vis spectroscopy. Microscopic examination used for studying insight information related to structures and physiology for fungi [11]. Macroscopic tool is used for identification of Aspergillus species and microscopic characteristics such as conidial color, colony diameter, growth rate, color of conidia and texture [12]. FTIR-ATR

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spectroscopy has high quality properties such as rapid scanning, extra sensitivity, inexpensive, that have been successfully used to characterize chemical composition of fungi samples [13,14]. It does not take more time to identify and characterize the biological samples such as fungi, and it needs a small quantity of fungi sample about 15 mg of sample in powder form. FTIR spectrum showed high accurate results for the identification and characterization of Aspergillus at the genetic level through the "molecular fingerprint" of each sample. The infrared radiation fall on the sample, some of them are absorbed and some of them carry out, the absorbed radiation energy that interacts with the molecular bonds vibrational and functional groups found in the chemical components of the fungi such as fatty acids, proteins, polysaccharides, carbohydrates, nucleic acids and aromatic compounds, which then showed specific wave numbers to form characteristic spectra for each sample of Aspergillus [15]. Raman spectroscopy has been widely used to study the biochemical composition and its analytical tool for rapid characterization and identification of microorganisms. An advantages of the Raman spectroscopic method is that it is fast, specific, a non-destructive for microbial analysis and an extended analytical method [16]. When we have been used Raman spectroscopic method, we did not need to add chemicals dyes to fungi samples for identify and classify them by that method. Raman spectroscopy is easy to use, its highly molecular specific and it gives wide information about chemical composition content of microorganisms such as fungi [17,18]. Raman spectra of biological molecules has been complex peaks because of fluorescence, therefore the fluorescence peaks has been removed from spectra by using first derivative spectra [19]. The π–π_* electronic transition of the amino acids available in protein due to the absorption of ultraviolet radiation [20]. Aspergillus contain aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, they represented of protein and they have ability to absorb ultraviolet radiation [21].

In this study, we used the Aspergillus spores to identify and distinguish eleven different Aspergillus species, four *Aspergillus flavus* and two *Aspergillus awamori* using morphology (MP), FTIR-ATR spectroscopy, Raman spectroscopy (RS) and UV–Vis spectroscopy (UV–Vis). We obtained similar results using those four methods, because each sample has a distinct chemical composition from the other sample through the spore. The advantages of Raman spectra are that for biological samples, it avoids infrared water absorption and it has non-broad spectral bands [22] when compared to IR spectra for same samples. IR spectra and Raman spectra generate specific spectra representing protein, lipids, polysaccharides, carbohydrates and nucleic acids of biological molecules such as Aspergillus species which provide information related to molecular structure and chemical information about fungal mass.

2. Material and methods

2.1. Collection of samples

Infected fruit samples were collected from different marketplaces of Aurangabad, Maharashtra state, India. Infected fruits *viz.* which include pomegranate, orange, papaya, tomato, grapes yellow, melon, mango, guava and lychees were collected in separate polythene bags. They were observed in laboratory. Primarily observations were made by preparing slides of fungal parts presents on the infected leaves and bulbs of onions.

2.2. Media preparation

Potato Dextrose Agar (PDA) Peeled Potato-200 g, Dextrose-20 g, Agar-20 g and distilled water -1000 ml, pH-6.5 Peeled potatoes were boiled until soft and pass through muslin cloth. Then dextrose was added in it. Later on in this solution agar was added, and final volume of solution was made up to 1000 ml and pH was adjusted to 6.5. Method of preparation: Irish Potato (*Solanum tuberosum*) was peeled; required quantity (200 g) weighed, washed and cut in tiny cubes. It was then transferred into a pot containing one liter of water and placed on a bunsen burner to boil until soft enough to mash. After mashing, it was squeezed through a sieve to obtain the pulp which was transferred into a 1000 ml measuring cylinder, 20 g of dextrose was dissolved and added Thomas JV (2000).

2.3. Sample preparation

Aspergillus species inoculated in petri-dish plates of potato dextrose agar at 28 °C for one week. All fungi colonies identified using microscopic technique (labomed vision 2000). We harvested spores with a bacteriological loop from agar plates for each isolated and cultured again in 250 ml conical flask containing potato dextrose liquid medium at continuous monitoring conditions for 15 days in dark at 28 $\pm$ 2 °C, after that we filtered each mycelia and fungal spores sample through Whatman No. I filter paper, and washed with the solution of 100 ml sterile distilled water and 20 ml ethanol thrice. All samples were dried and used in powder form for FTIR and Raman spectroscopic characterization.

2.4. Morphological and identification of fungal isolates

Identification of fungal forms PDA medium was used to maintain stock cultures. Isolated fungal forms were identified on the basis of available literature, including manuals and monographs as "The genus Aspergillus" [23]. The morphological characteristics were done after one week of fungi cultivation. Macroscopic features are conidial color, diameter, observing growth rate control, colony reverse, smooth and texture of colonies. Microscopic features are conidiophores, stipes, ornamentation of conidia, branching of individual hyphae and length. The microscopic study is carried out by preparing the slide using lacto phenol blue cotton and observed under the optical microscope. The micro slides were prepared in Cotton blue stain and mounted with lacto phenol. Small tuft of the fungus usually with spore and spore bearing structure were transferred into the drop with the help of a flamed, cooled needle. The fungal material was teased using two mounted needles and the mold structures mixed gently in the stain. The cover-slip was placed over the fungal materials without air bubbles in the stain. The lacto phenol mounts were sealed around the edge of the cover-slip with the help of fresh nail polish. The microphotographs and micro measurements were done for every isolated fungal form fruit.

2.5. Microscopic (Labomed microscope-vision 2000) characterization

Optical microscope consists of eyepiece (WF10X), a light source (3 W LED) and quadrant revolving nosepiece with rubber grip were used for characterization of fungi directly by microscopic slides preparative techniques. We used digital camera Sony Cyber Shot W810, 20.1 M pixels for photographs of fungus.

2.6. FTIR-ATR spectroscopy

A Fourier transform infrared (FTIR) spectrometer (Bruker, Germany) was used to record the spectra of samples in 4000 and 500 cm^{-1} wave numbers region with an Attenuated Total Reflectance (ATR) at resolution of 4 cm^{-1} with 24 scans for each spectrum at room temperature, 15 mg quantity of samples in powder form were placed on the a transparent ZnSe crystal as homogeneous layer and dry for high absorbance. Spectra were recorded for all samples and smoothing by OPUS (Bruker) software.

2.7. Raman spectroscopy

Raman spectra were taken by Raman microspectrometer with Olympus microscopy using green laser light 520–570 nm, which

focused onto fungi sample on powder form. Raman spectral has been collected in the range 200–1800 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

2.8. UV–Vis spectroscopy

The UV–Vis spectroscopy measurements for fungi samples after preparation were performed by using a double beam UV–visible spectrophotometer (ELCO 210) in the range of 190–1100 nm with the resolution of 0.5 nm. For obtain homogeneous liquid and sample dispersion in the solution, we used magnetic stirrer. Typically 5 mg of the each Aspergillus sample powder was added to the solution has concentration 90% distilled water and 10% ethanol at 28 °C in 100 ml flask putting on magnetic stirrer plate and agitated at 1000 rpm.

3. Result and discussion

3.1. Morphological characterization of Aspergillus species

Fig. 1(A, B, C, D and E) shows the micromorphology and macro morphology of the 15 Aspergillus species growth on potato dextrose agar (PDA) after culturing for 7 days at 28 ± 2 °C. In this study all Aspergillus species moderate to rapid growth on PDA. ***Aspergillus flavus***, isolates acquired the white color of the mycelia, the color of the colonies is green, reverse side colorless to yellow, soluble pigments, conidial head was greyish green.Conidia size range was between 3.5 and 5 μm; globose; smooth to finely rough and yellow green color as shown in Figure (for flavus) [24–26]. ***Aspergillus oryzae*** isolates acquired the white color of the mycelia, the color of the colonies is olive green, reverse side white to slightly gray, soluble pigments, conidial head was yellow-green colony was globose to sub-globose vesicles, vesicle diameter and shape; 18–36 μm; radiate, and ornamentation almost smooth.

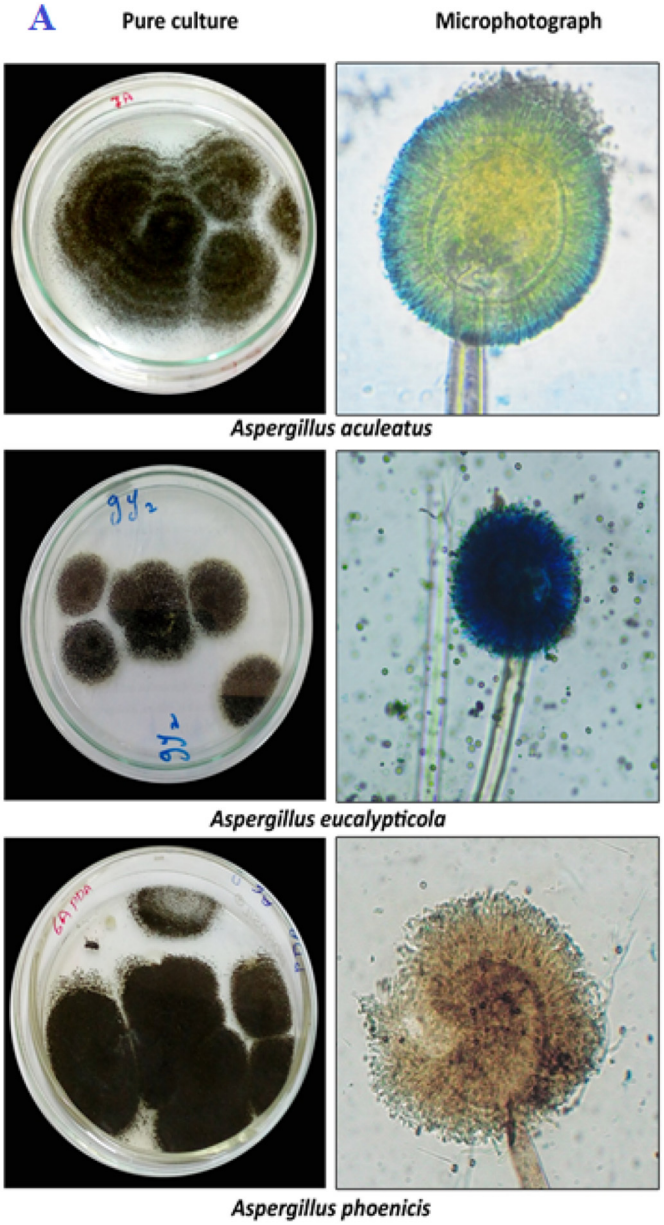
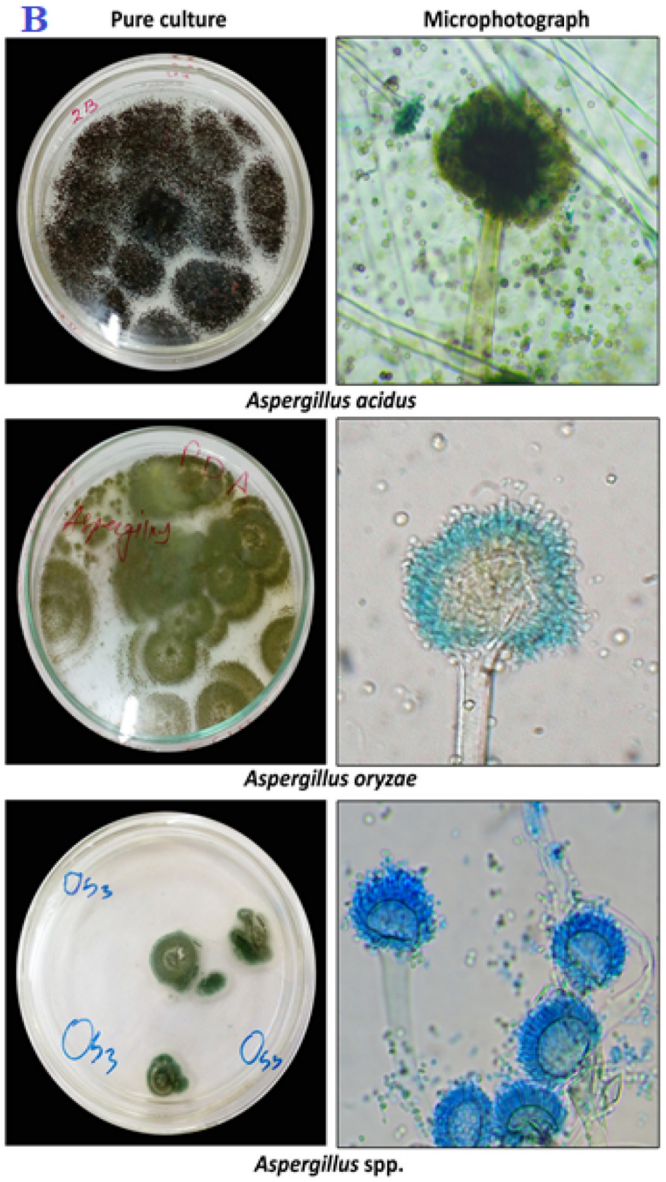


Fig. 1. A, B, C, D and E observation of Aspergillus species colonies and microscopic morphologies: (A) three species (Aculeatus, Eucalypticola and Phoenicis); (B) three species (Acidus, Oryzae, A. Spp.); (C) three species (A. Spp., Awamori (Papaya) and Awamori (Orange); (D) three species (Carbonarius, Brasiliensis and Flavus (Guava)); (E) Flavus (Melon), Flavus (Mango) and Flavus (Papaya) Colonies after incubation for 7 days at 28 ± 2 °C on PDA and conidiophores and conidia.



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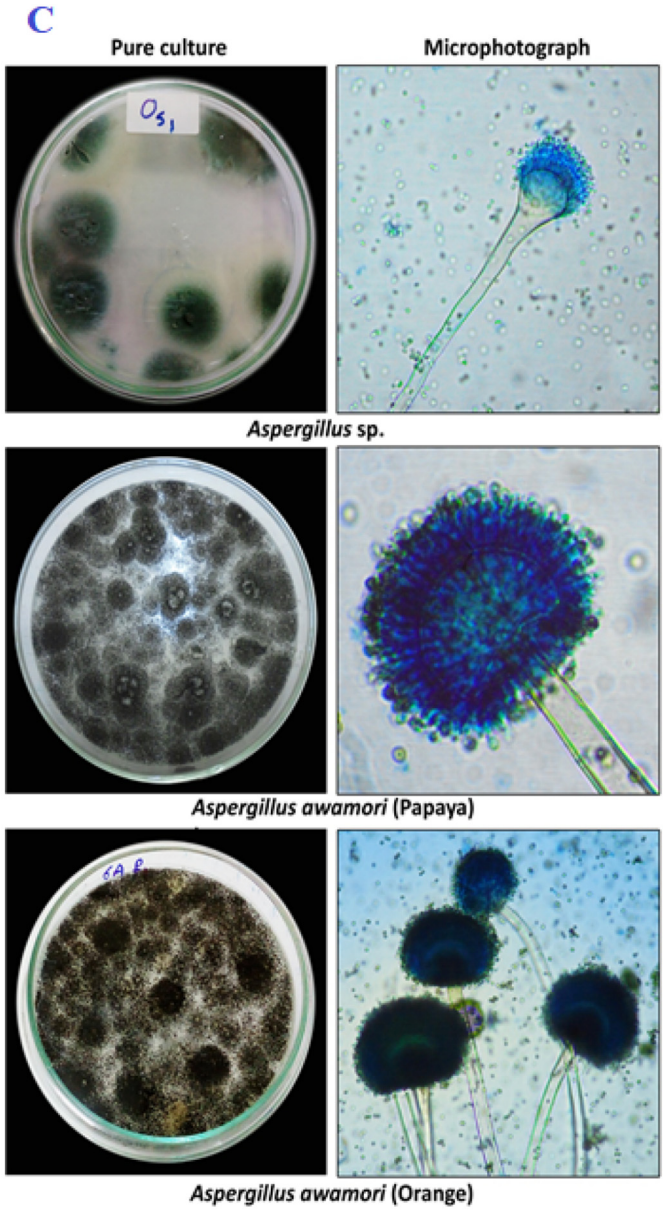
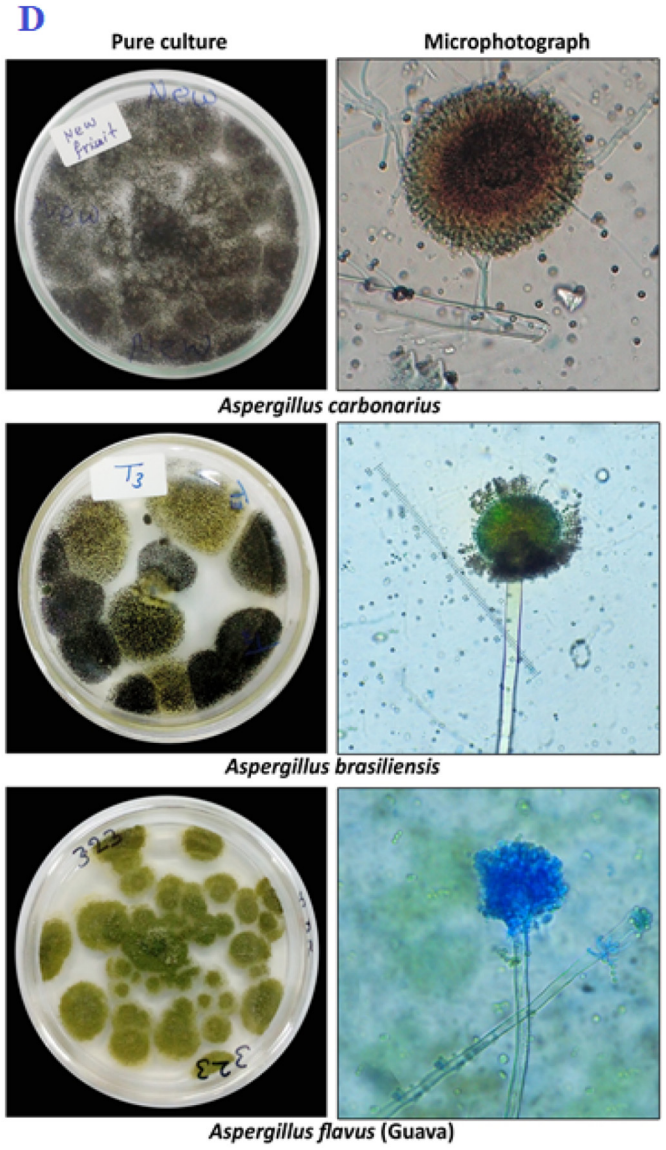


Fig. 1 (*continued*).

Aspergillus brasiliensis*, *Aspergillus carbonarius* and *Aspergillus phoenicis*,** have color of the colonies is dark-brown-to-black, reverse side colorless to yellow, biseriate conidiophores, conidia were spherical vesicles and lightly and soluble pigments [27] ***Aspergillus carbonarius has conidia were rough and split into columns with age and Conidia size ranged between (6) 7–10 (11) μm, globose, with rough walls and black [26]. ***Aspergillus brasiliensis*** has Conidia sizes ranged 3–4.5 μm; rough and brown. Diagnostic feature; the colony had distinct sporulation rings on PDA and long stipe [26]**.** ***Aspergillus phoenicis*,** conidial heads color from light tan to black and sizes ranged 3.0 to 3.5 μm. ***Aspergillus awamori***, color of the colonies was black in color with white margins, reverse side slightly yellowish, conidiophores were vesicles globose and conidial head was black, it having smooth walls [28]. ***Aspergillus acidus* and *Aspergillus eucalypticola***, color of the colonies is black, reverse color cream yellow, conidiophores biseriate with globose vesicles and conidia globose has brown color, smooth-walled to roughened and coarsely roughened respectively [29]. ***Aspergillus acidus*,** conidia globose, 3–4 μm, brown, smooth-walled to roughen. ***Aspergillus aculeatus*,** has color of the colonies is black, reverse color light yellow, slightly pigmented, sporangium was spherical and conidia has brown color and size 3.5 μm–4.5 μm [30]. ***Aspergillus spp.*** (1), color of the colonies is greenish gray color reverse colorless sporangium was spherical and conidia has white with green margins color. ***Aspergillus spp.*** **(2)**, has colonies with greenish gray color, reverse color was colorless, sporangium was spherical, conidial head was greenish color and lightly pigments.

3.2. FTIR-ATR spectroscopy analysis

Optical microscopy technique used to classify the types of Aspergillus by observing spores and conidia those are present in the internal structure of each Aspergillus species isolated from fruits. Aspergillus species have small differences between them, this is why it is not easy to classify them using one technique. Therefore, we used FTIR-ATR spectroscopic technique to confirm the results obtained by using optical microscopic examination. The infrared absorption spectra of 15 samples of different Aspergillus species (four identical *A.flavus* and two *A. awarmori*) in the wavenumber region 3800 cm^{-1}–600 cm^{-1} are reported in Fig. 2. The FTIR spectra in the high wavenumber region (3600–3350 cm^{-1}) represents water absorption spectrum [31]. Water



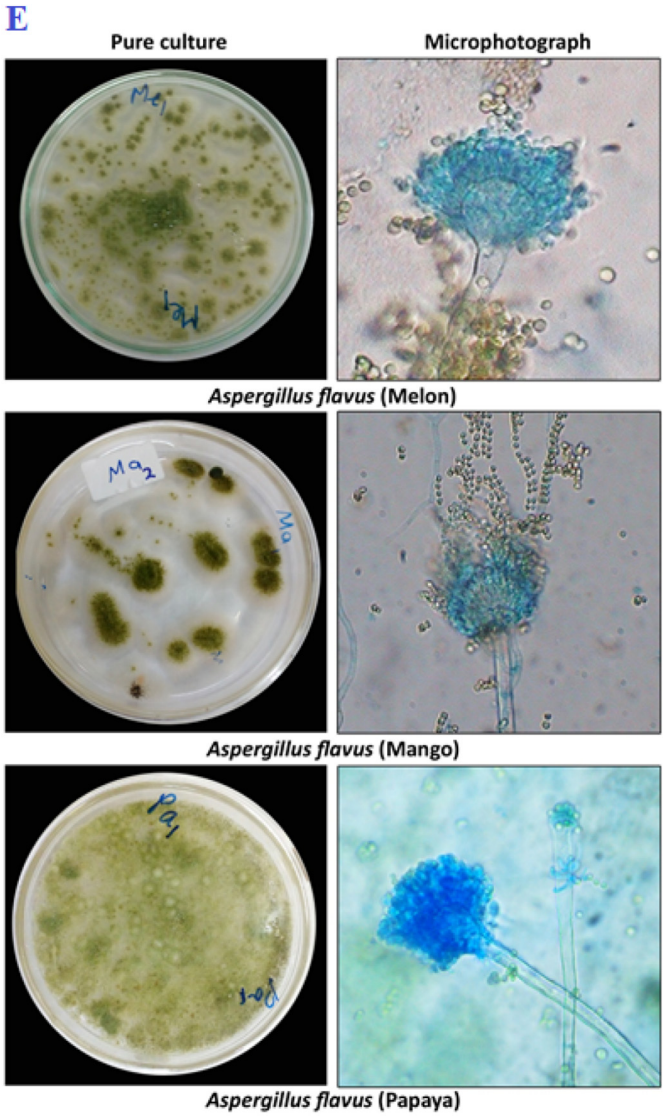


Fig. 1 (*continued*).

absorption bands of fungi are characterized by O—H stretching bands of hydroxyl group at 3600–3350 cm^{-1}, it is common region for all fungi. Fig. 2 shows the bands 3286 cm^{-1} and 3257 cm^{-1}, which are attributed to protein content (N—H stretching of Amide A group) [8]. The N—H stretching band is observed at different wavenumber for all studied species. The shifting in N—H stretching absorption peak is less in case of four identical *A.flavus* and two identical *A.awarmori* species. The N—H stretching absorption peak is appeared at around same wavenumber. It is also observed that the intensity (indicates the concentration of proteins) of N—H stretching band of four identical *A.flavus* and two identical *A.awarmori* species are almost same.

Wave numbers between 2950 cm^{-1} and 2800 cm^{-1} were represented spectral region of the fatty acid (phospholipid) dominated by C—H stretching aliphatic groups of fungi [14]. The infrared absorption spectra of fatty acid which has observed in the range 2924 cm^{-1}–2853 cm^{-1}. The C—H stretching absorption peak for all four identical *A.flavus* species is observed at 2923 cm^{-1} whereas for *A.awarmori* species it appeared at 2920 cm^{-1} and 2873 cm^{-1}. The intensity of C—H stretching absorption peak is found to be nearly same for identical *A. flavus* and *A.awarmori* species whereas it is different for remaining species.

Carbon dioxide (CO_2) group was observed for all Aspergillus species except *A. phoenicis* and *A. oryzae* in the range 2365–2335 cm^{-1} [32]. So *A.phoenicis* and *A. oryzae* are used in the food industry [7], because of the presence of CO_2, Aspergillus species have the potential to cause infection in humans, plants and animals. The C═O stretching peak for *A. awarmori* species are observed at 2350 cm^{-1} whereas it is at around 2335 cm^{-1} for *A.flavus* species. The C═O stretching peak for identical *A.flavus* and *A.awarmori* species overlap whereas the wavenumber and intensities are found to be different for remaining Aspergillus species.

Proteins and peptides have spectral region with absorption bands at 1650 cm^{-1}- 1500 cm^{-1} [33], this region dominated by amide I and amide II bands of proteins for Aspergillus types of fungi. Amide *I* groups represented by carbonyl [34] and C—N stretching band [7] and appeared in the region 1639 cm^{-1}–1619 cm^{-1}. Whereas amide II appeared in the range 1554 cm^{-1}- 1543 cm^{-1} and represented by C—C, C—N stretching, N—H and C—O bending vibrations [8,34].

It is observed that the amide I and amide II peaks for identical *A. flavus* species appeared at around 1637 cm^{-1} and 1545 cm^{-1}, respectively. Whereas in case of *A.awarmori* they appeared at around 1620 cm^{-1} and 1551 cm^{-1} respectively. It is also observed that the peak intensities are almost same for similar species. The absorption peak (amide I and amide II) intensities and wavenumber are found to be different for different species because of each sample contains a

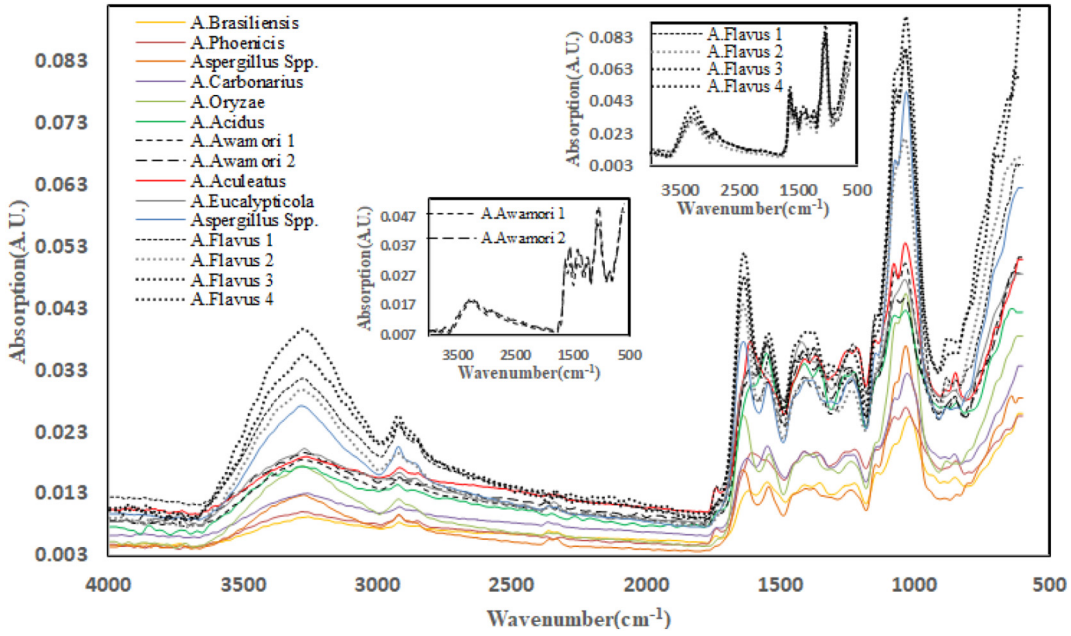


Fig. 2. Mid infrared spectra in the region 600 cm^{-1}–3700 cm^{-1} of 15 different species of Aspergillus: Brasiliensis, Phoenicis, A. Spp., Carbonarius, Flavus-1, Flavus-2, Flavus-3, Flavus-4, Acidus, Awamori-1, Awamori-2, Aculeatus, Eucalypticola, A. Spp. and Oryzae.

specific amount of protein. In case of *A.Aculeatus* and *A.Phoenic* amide II bands of protein are not appeared in their IR spectra.

Mixed region 1500 cm^{-1}–1300 cm^{-1} represents the lipids content, protein (amide III) polysaccharides and bioactive component triterpene compounds [14,35,36]. O—H bending peaks which are related to absorption of polysaccharides and bioactive component triterpene compounds (CH_2 = CH-CH_3) are observed for *A.Brasiliensis* (1411 cm^{-1}), *A.carbonarius* (1412 cm^{-1}), *A.phoenicis* (1412 cm^{-1}), *A. acidus* (1412 cm^{-1}) and *A. Awamori* (1416 cm^{-1}). The absorption region 1422 cm^{-1}–1401 cm^{-1} represents the C—N stretching which are related to protein group and observed for four identical *A. flavus* species (1401 cm^{-1}), Aspergillus Spp. (1404 cm^{-1}), A.Oryzae (1406 cm^{-1}), *A. Aculeatus* (1410 cm^{-1}), for *A. eucalypticola* (1422 cm^{-1}).

O—H bending of polysaccharide absorption bands are observed in the range 1382 cm^{-1}–1344 cm^{-1} for all Aspergillus species (except *A. eucalypticola*). Phosphate groups (nucleic acids, phospholipids), carbohydrates cell walls of fungi, C—O and C–O–C absorption peaks of the cell wall polysaccharides, they have absorption bands in the region 1200 cm^{-1}–900 cm^{-1} [15,31,33,37–42] and for all studied Aspergillus species they are appeared in the region 1260 cm^{-1} -1020 cm^{-1}. IR spectra showed an absorbance in the region 1260 cm^{-1}–1224 cm^{-1} for Amide III [35], *A.Acidus* is observed at higher wavenumber whereas *A. Brasiliensis* is at lower wavenumber. For *A.flavus* and *A. Awamori* it is observed at around 1238 cm^{-1} and 1244 cm^{-1}, respectively.

Fig. 2, represents C—C, C—O and C-O-C [38,39] absorption peaks of the cell wall polysaccharides observed in the region 1146 cm^{-1}–1140 cm^{-1}. From this figure it is observed that the absorption peaks of the cell wall polysaccharides appeared for *A.Carbonarius* (1140 cm^{-1}), *A.Brasiliensis* (1141 cm^{-1}), *Aspergillus Spp.* (1146 cm^{-1}), *A.Oryzae* (1147 cm^{-1}) and *A.Aculeatus* (1149 cm^{-1}), the intensity is more for *A.Oryzae* and less in case of *A.Brasiliensis*.

The absorption peak in the range 1070 cm^{-1}–1076 cm^{-1} for the P═O symmetric stretching phosphate groups of DNA, RNA, phospholipids and nucleic acid observed for all fungi species except *A. acidus* (1017 cm^{-1}) and *A.brasiliensis* (1020 cm^{-1}) [15,33,39,40]. Both *A. acidus* and *A.brasiliensis* species possess characteristic properties for this reason they are used in industry for the production of xylanase [40]. For *A.flavus* and *A. Awamori* absorption peak for the P═O symmetric stretching observed at around 1070 cm^{-1} and 1076 cm^{-1}, respectively. Finger print region in the range 900 cm^{-1}–700 cm^{-1} [41–45], which have less intense bands and useful for the identification of polysaccharide of Aspergillus species, this region can be observed due to the contributions of sugar–phosphate chain functional group of DNA/RNA [46].

3.3. Raman spectroscopy analysis

Raman spectra recorded for eleven samples of Aspergillus in the rang 1000 cm^{-1}–1700 cm^{-1} and 400 cm^{-1}–1000 cm^{-1} represented in Figs. 3 and 4, respectively. Raman spectra provide us important biochemical information related to the internal and molecular structure of the fungi species and their fungal mass. In the Raman spectra, specific peaks are observed for all fungi samples due to the biological molecules such as protein, lipids, saccharides carbohydrates and nucleic acids present in it. Raman spectral region 1639 cm^{-1}–1609 cm^{-1} and 1576 cm^{-1}–1547 cm^{-1} represents the C—N, C—C stretching and N—H deformation groups [19,22] of Amide I and Amide II, respectively, which indicates the presence of proteins (Fig. 3a and b). It is observed from Fig. 3a and 3b that the peak intensities are different for different species which indicates that the amounts of protein components are different in different species. Lipid contribution was observed at the range 1469 cm^{-1}–1402 cm^{-1} for all species of Aspergillus due to group of CH_2 deformation [19,22,47]. Intensities of peaks are observed different for different species which indicates the amount of lipid are different in different species. The Raman peaks in the region 1386 cm^{-1}–1323 cm^{-1} represents the glycogen (polysaccharide of glucose) [22,48], which exhibits due to deformation vibrations of CH_2 and observed for all species. Raman bands at 1302, 1313, and 1314 cm^{-1} indicates that contribution of amide III [47] for proteins in, *A. A.eucalypticola*, *A.aculeatus* and *A. phoenicis*, respectively. Raman spectrum of all Aspergulls species exhibits bands in the regions of 1287 cm^{-1}–1214 cm^{-1} which can be assigned to amide III band components of protein [19,47,49]. High intensity of Raman peak are observed at 1279 cm^{-1} and 1276 cm^{-1} for some species, which indicates that the protein content is different in different species. The region 1158 cm^{-1}–1130 cm^{-1} corresponding to C—C and C—O stretching groups for polysaccharides [50] and observed for all species. The peaks in region 1086 cm^{-1}–1063 cm^{-1} were assigned to O–P–O (DNA) [47,51], observed for all species, but intensities of each peak is different for different species. Which indicates that

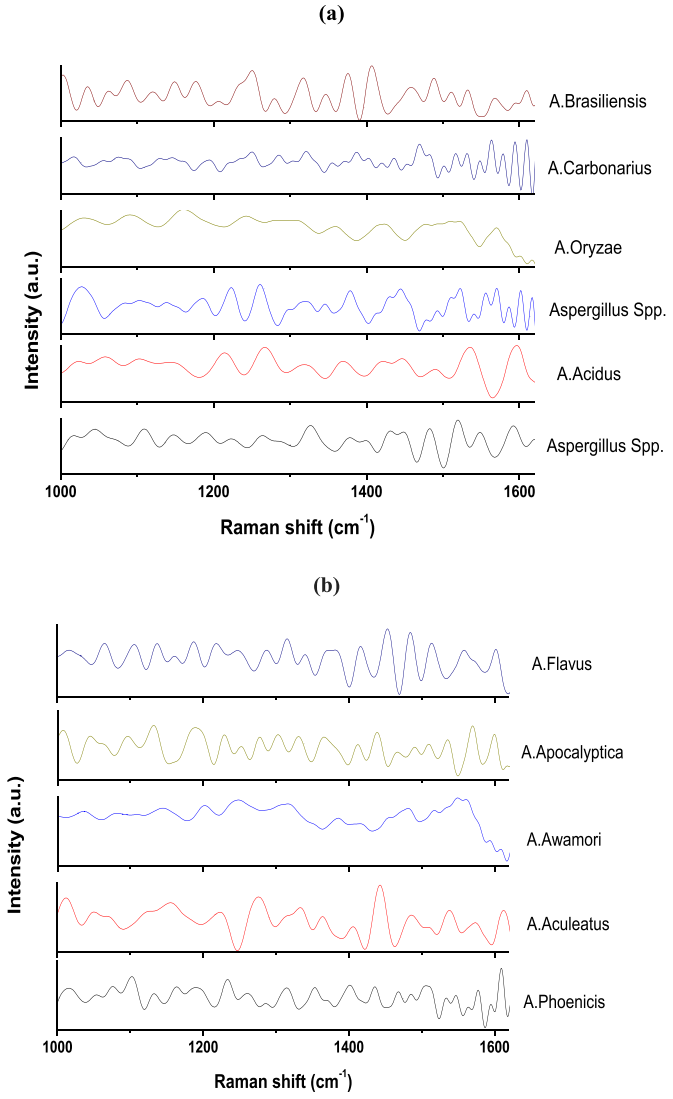


Fig. 3. (a) Raman spectra of A. spp., A. acidus, A. spp., A.oryzae, *A. carbonarius* and A. brasiliens in the range 1000–1700 cm^{-1} (b) Raman spectra of A. phoenic, A.Aculeattus, A. Awamori, A.eucalypticola and *A. flavus* in the range 1000 cm^{-1}–1700 cm^{-1}.

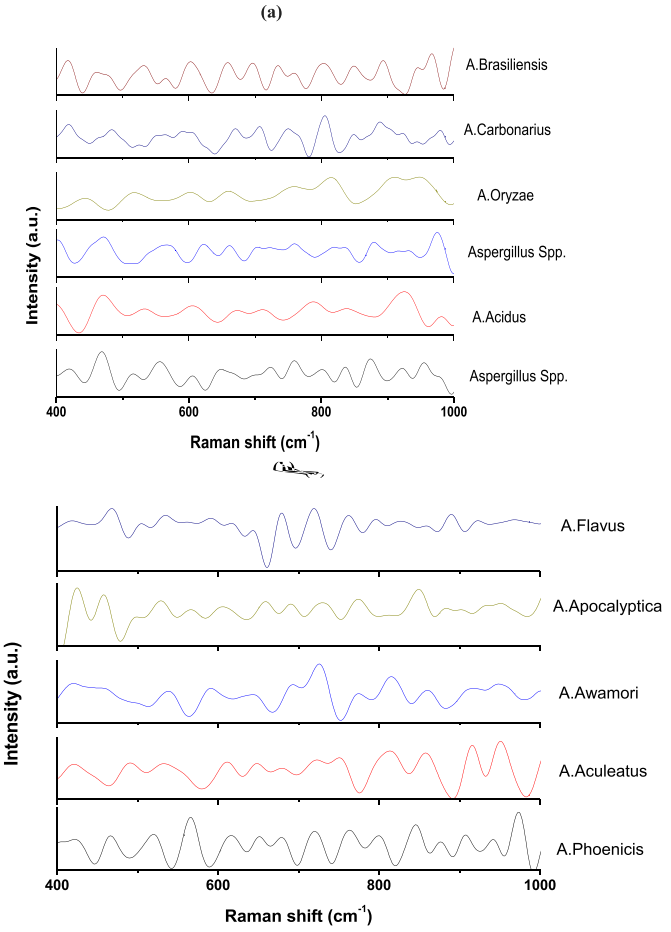


Fig. 4. (a) Raman spectra of A. spp., A. acidus, A. spp., A.oryzae, *A. carbonarius* and A. brasiliens in the range 400–1000 cm^{-1} (b) Raman spectra of A. phoenic,A.Aculeattus, A. Awamori, A.eucalypticola and *A. flavus* in the range 400 cm^{-1}–1000 cm^{-1}.

each sample has a different amount of nucleic acids and helps us to characterize and distinguish each Aspergillus species.

The lower wavenumber region 999 cm^{-1}–902 cm^{-1} and 896 cm^{-1}–840 cm^{-1}, assigned to C-O-C stretching and C—C stretching in carbohydrates [49,52] and observed for all fungi samples. From Fig. 4, it is observed that six samples have high intensity in this region such as *A. spp.*(1), *A. spp.*(2), *A.oryzae*, *A.brasiliens*, *A.aculeatus* and *A.phoenic* and the remaining five samples in this region have low intensity. In addition to that some peaks did not appear in *A. acidus*, *A. carbonarius*, *A. Awamori*, *A. flavus* and *A. Eucalypticola*, this is because of the species studied are not same although they are from the same family of Aspergillus. Bands for the nucleic acid are appeared in the regions 670 cm^{-1}–622 $cm^{-1}$and 835 cm^{-1}–702 cm^{-1}, which represented of Adenine, Cytosine, thymine, phosphodiester band in DNA and RNA [16,51,52], (Fig. 4a and b). The peaks at the region 567 cm^{-1}–461 cm^{-1} indicates carbohydrate and assigned to the deformation (COC) glycosidic ring, deformation (CCC) and chitin [19,22,50–52] which appeared in all species. The polysaccharide beta (1,3)-D-glucan (range 424 cm^{-1}–413 cm^{-1}) [51], observed in all species. The *Aspergillus oryzae* has contains a large amount of polysaccharides as it has high intensity peaks observed in this region 424 cm^{-1}–413 cm^{-1}, so it can be used for the production food ingredients and enzymes. It is interesting to observe from the Fig. 5(a) and (b), that the Raman spectra for four identical *A.flavus* and two identical *A.awamori* are almost identical.

3.4. Analysis of UV spectra for Aspergillus species

The recorded UV–Vis absorption spectra for fifteen Aspergillus species which includes three regions: UV-A (315–400 nm), UV-B (280–315 nm), UV-C (100–280 nm) [53,54] and also visible absorption region (400–700 nm) [53,55,56] reported in Fig. 6. It is found that the UV–Vis absorption peaks are appeared in the range 256 nm–423 nm. From this figure it is observed that UV-C radiation absorption peaks are appeared for all studied species except *A.Brasiliensis* species. Only one UV-C radiation absorption peak is observed for *A.Eucalypticola* (256 nm), A.Oryzae (256 nm), *Aspergillus Spp.* (256 nm), *A.Acidus* (257 nm), four identical *A.Flavus* (258 nm), two identical *A.Awamori* (258 nm), and A.Carbonarius (262 nm). Whereas two absorption peaks for *A.Aculeatus* at 264 nm and 278 nm, and three peaks for *A. Phoenicis* at 221 nm, 264 nm and 277 nm are observed. Only one species (*A.Phoenicis*) has absorbance peak at 221 nm, this peak may be due to absorption by amide bond [21].

UV-B radiation absorption peak at 282 nm observed in the intensity order for *A.Brasiliensis*, *A.Carbonarius*, two identical *A.Awamori* and *A. Eucalypticola* (282 nm). Whereas *Aspergillus* Spp. *is* observed at 285 nm. These UV-B radiation absorption peaks indicates the presence of tyrosine and tryptophan of the proteins [21].

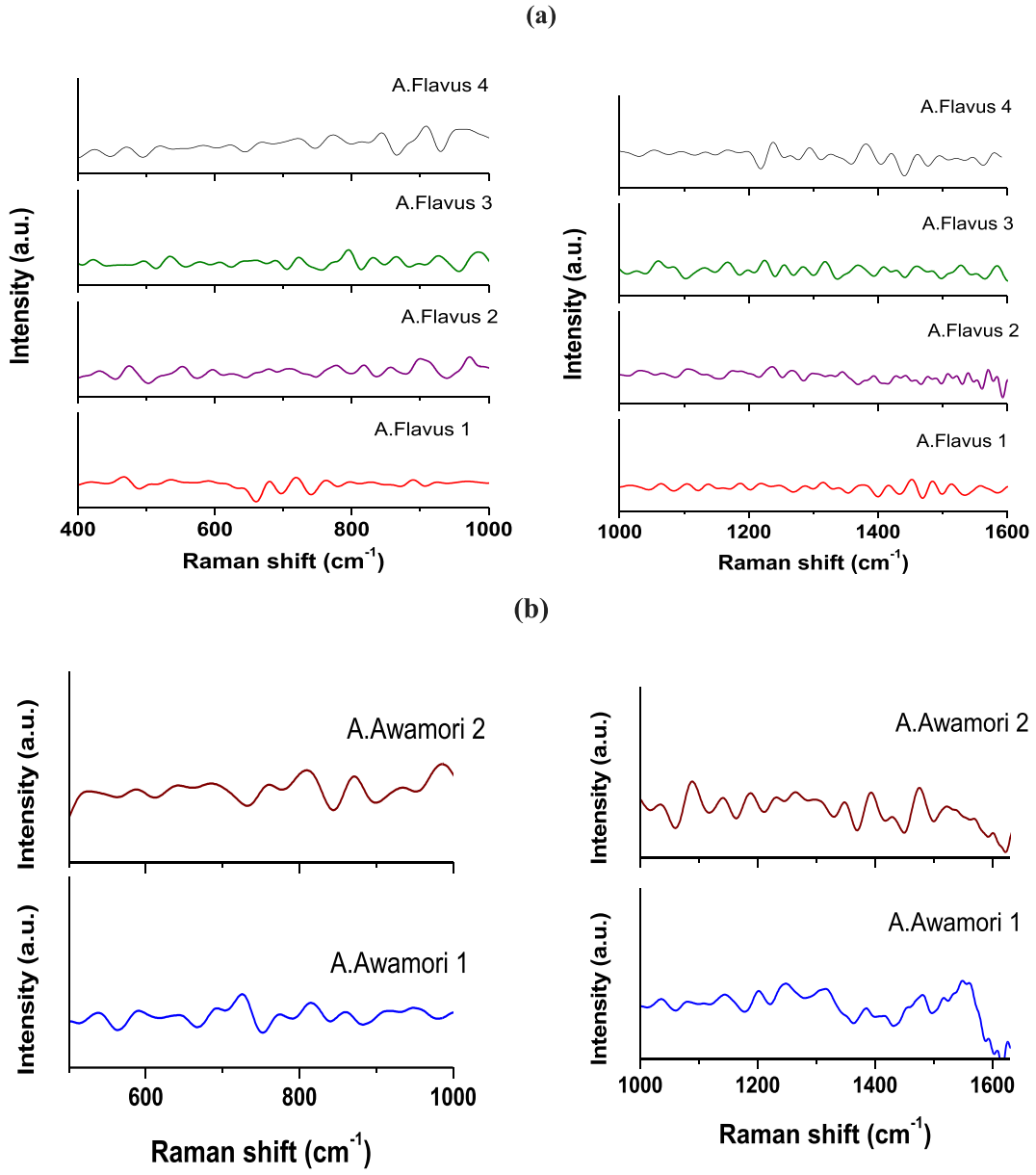


Fig. 5. (a) Raman spectra of four *A. flavus* in the range 400 cm^{-1}–1650 cm^{-1} (b) Raman spectra of two A. awamori in the range 400 cm^{-1}–1650 cm^{-1}.

From Fig. 6, it is also observed that the species such as *A.Oryzae*, *Aspergillus Spp*., *A.Carbonarius*, and *A.Acidus*, have weak UV-A radiation absorbance at 397 nm, 383 nm, 372 nm and 371 nm, respectively. These absorption peaks indicates the presence of cytosine and thymine of DNA [57].

Aspergillus species have different pigments and they show absorption peaks in the visible range 400–700 nm of UV–Visible spectra [58,59]. Biological pigments including melanin produced by species of Aspergillus absorb all visible wavelength, so we used UV–visible spectrophotometric to analysis and detect dark color of the fungi pigments [58]. In the visible region 401 nm–423 nm, species such as *A.Brasiliensis*, *A.Aculeatus and A.Phoenicis* have high intensity peaks at 423 nm, 415 nm and 412 nm, respectively. These peaks indicates the presence of pigments [55,57,60,61] for mycelium and spores of species. From fig. 12, it is observed that nature of the spectra for identical species are same. Therefore, UV–Vis spectroscopy can be used as a method to identify and distinguish Aspergillus species, depending on the amino acids (tyrosine and tryptophan), presence of cytosine and thymine of DNA and presence of pigments in different colors that define the different Aspergillus species.

4. Conclusion

Aspergillus species of fruit rot fungi have been characterized and identified using FTIR-ATR spectroscopy, Raman spectroscopy, UV–Vis spectroscopy and microscopy. From this study it is found that each species has a special feature and reflects in their FTIR, Raman and UV–Vis spectra. Results showed that spectroscopic methods are consonant with morphological characteristics for identification and characterization of studied species. It is found that four identical *A.Flavus* and two identical *A.Awamori* species have similar FTIR, Raman and UV–Vis spectra, whereas it is different for different species. All the methods used in this study are complementary in the presentation of biochemical components information about the types of Aspergillus and their classification. These methods are reliable methods for rapid and accurate identification and discrimination between different species. Some of

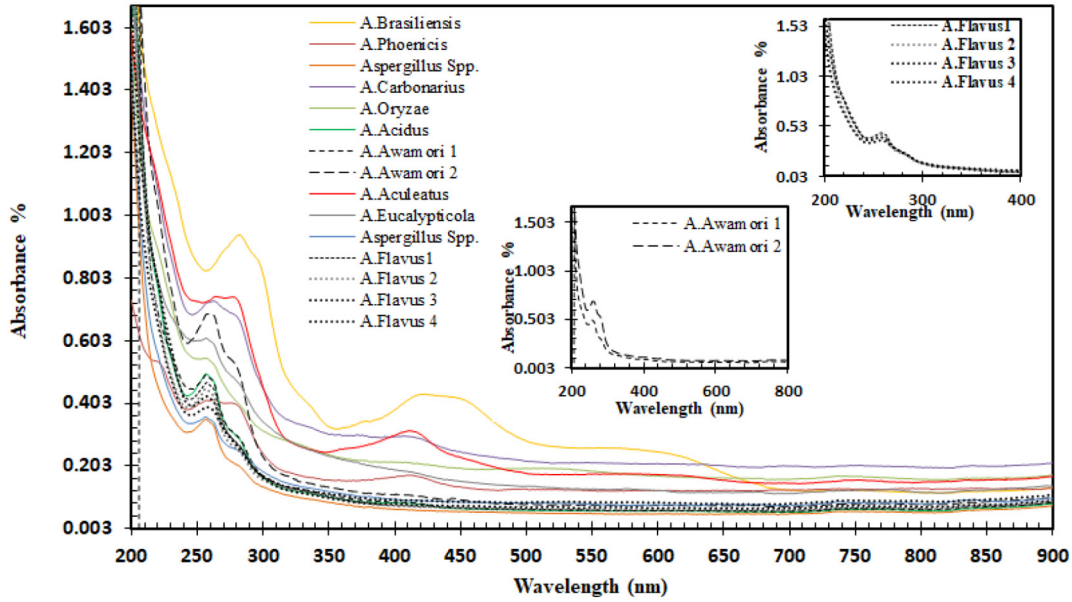


Fig. 6. UV–Vis absorption spectra recorded from the Aspergillus: Flavus-1, Flavus-2, Flavus-3, Flavus-4, A. Spp., Phoenicis, Acidus, Oryza, Eucalypticola, Awamori-1, Awamori-2, Carbonarius, Aculeatus, and Brasiliensis.

the species contain a bioactive components and these bioactive compounds are important in the pharmaceutical industry.

CRediT authorship contribution statement

All authors listed have made a substantial, direct and intellectual contribution to the study, manuscript preparation and approved it for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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