KAVAKA 60(2): 49-59 (2024) DOI: 10.36460/Kavaka/60/2/2024/49-59

Insight to the Degradation of Shrimp Shell Waste by *Aspergillus fumigatus* Isolated from Mallipattinam Southeast Coast of Tamil Nadu

B. Deepa*[1], K. Kanimozhi[1] V. Ambikapathy[1], A. Panneerselvam[1] and N Thajuddin[2]

[1]*PG & Research Department of Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur, Tamil Nadu, India (Affiliated to Bharathidasan University, Tiruchirappalli-24, Tamil Nadu, India).*

[2]*B. S. Abdur Rahman Crescent Institute of Science & Technology, Vandalur, Chennai - 600 048, India.*

**Corresponding author Email: deepa.micro85@gmail.com*

(Submitted on April 1, 2024; Accepted on June 2, 2024)

ABSTRACT

In the present study, to isolate *Aspergillus fumigatus* fungi from soil in order to potentially utilize them for the degradation of shrimp shells and spectroscopic (GCMS, FTIR) investigation of the chitnase enzyme. Soil samples were taken from two distinct locations of east and west at the Mallipattinam southeast coast of Tamilnadu. Using standard mycological techniques, the 25 strains were isolated and identified from the soil samples (cultural and morphological traits). Shrimp shell waste was used to treat the isolated fungi in order to break them down and produce chitinase. It was established what the activity ratio, weight percentage, and enzyme activity were. The fungi with the greatest documented chitinolytic activity were *Aspergillus fumigates, A. terreus, A. niger, A. flavus, Pencillium citrinum*, and *Trichoderma viride*. Proteins, amino acids, and carbohydrates were detected by the biochemical analysis of waste decomposed by shrimp shells. The bioactive compounds (23) were identified by GC-MS analysis, including Tri-O-Trimethylsilyl, N-Pentafluoropropionyl derivative of terbutaline and 4-(Dimethylamino) Azoestrone 3-Methyl Ether. Additionally, the FTIR examination of the degraded shrimp waste revealed the presence of amine and OH groups. It was determined that, in comparison to the other fungal groups, *Aspergillus fumigatus* produced the highest level of chitinase enzyme. The high activity of isolated cultures in breaking down shrimp shell waste points to a wide range of possible applications for these fungi in eco-friendly methods of extracting chitinase from wastes high in shrimp shell degraded waste.

Keywords: Chitinase, Biodegradation, GCMS, FTIR, Shrimp shell waste

INTRODUCTION

The largest class of crustaceans, the Malacostraca, includes shrimp. Reports from FAO 2020 indicate that each year, almost 9.3 billion tonnes of crustaceans shrimp, prawns, and lobsters were produced. Asia's entire production of coastal crustaceans, both marine and inland, accounted for 89.6% of the global total. The shrimp farming and processing industries produce large volumes of waste from shellfish due to demand for their consumption (FAO, 2020). These wastes must be valorized to create value-added products in order to prevent contamination of the environment. The head and the shell, or body carapace, make up about 45–48% of the shrimp that are thrown away as trash (Sachindra and Bhaskar, 2008). A portion of the shrimp waste generated is utilized locally for feeding animals or as food in aquaculture, but the bulk is dumped in landfills (Evers and Carroll, 1998), the open ocean (Xu *et al*., 2013), or is burned (Kreag and Smith, 1975). Due to the production of odors (gases, fumes, and dust) and the wastes' rapid decomposition, which spreads infectious diseases brought on by rodents, flies, mosquitoes, and other pests and pollutes the environment, these shrimp waste disposal landfills are closing. Since these discarded shrimp shell wastes are rich in protein, chitin, lipids, and colors and have a high commercial value, creative and hardworking ways for using them are progressively emerging (Benhabiles *et al*., 2013).

Microorganisms are an excellent way to remove chitin from shrimp shells, and the resulting chitin powder has applications in both the food industry and biomedicine (Arbia *et al*., 2013). Glucosamine hydrochloride, chitosan, and chitin have been produced from shrimp shell wastes by the Central Institute of Fisheries Technology (CIFT), Kerala. Due to its high immunogenicity, chitin derived from

shrimp shells was created and patented as a nasal spray to treat hay fever. According to Dutta *et al*. (2002), these biomaterials have found use in the paper, textile, wastewater treatment, chromatography, and biomedical industries. According to Rengga *et al*. (2018), chitosan derived from deacetylated shrimp shell waste has been investigated as a matrix controller for the slow release of NPK fertilizers in agriculture. Colorants, medications, and nutraceuticals are all made from chitinase that is extracted from prawn or shrimp shells. In the Asian countries, the shrimp shell concentrates are an integral part of their food, as additives in soups, sauces, and stock concentrate. The sterile, autoclaved shellfish can be divided into liquid and solid parts. The liquid hydrolysate can be utilized as a biofuel or as a fertilizer, and the solid fraction can be utilized to recover chitin and chitosan (Das *et al*., 2013). Due to their high catalytic activity, shrimp shells have been reported by (Yang *et al*., 2009) to be an effective and environmentally friendly catalyst for the synthesis of biodiesel. This paper's importance lies in its evaluation of ecologically sound and sustainable techniques for valuing shrimp shell trash. The initial demonstration of the chemical approaches is to comprehend the advantages of the eco-friendly techniques used in the value-adding of shrimp shells. From this angle, the goal of the current investigation was to identify a strong chitinolytic fungus that could effectively break down shrimp shell debris. the impact of soil fungus on the separation of chitinase from the degraded shrimp shell waste and the degradation of shrimp shell waste. By using GCMS and FTIR to analyze hydrolysate and degraded shrimp waste, the hydrolytic efficacy of chitinase was further assessed.

MATERIALS AND METHODS

Isolation of fungi

Two distinct locations inside the shrimp waste disposal region of east and west at the Mallipattinam landing hub, which is located on the southeast coast of India (Mallipattinam – Lat. 10°16′50.02 N; Long. 79°19′1.13 E), were used to gather soil samples. Potato dextrose agar (PDA, Himedia) plates were used to plate the 1 g soil sample after it had been serially diluted. For seven days, the plates were incubated at 30 °C. After the incubation period, the plates were closely inspected to look for evidence of fungal development. Any fungal growth that was noticed was further subcultured and kept at 4°C on PDA slants. Using references to Barron (1968), Ellis (1976), and Domsch and Gams (1972), the physical traits of the fungal isolates, such as morphology, mycelia structure, and spore development, were used to identify them.

Collection of shrimp shell waste

The shrimp shell waste was collected from Kumbakonam, Thanjavur, Tamilnadu, India is home to a small fish market. After obtaining the shells, they were cleaned under running water to get rid of any remaining shrimp and other contaminants. Following that, distilled water was used to wash and dry the shells. Ultimately, a grinder was used to break the dry materials into tiny pieces of 2-3 mm.

Isolation of Chitnolytic microorganisms

The soil samples were serially diluted and then put on chitin agar, which was made up of 2% agar, 0.5% yeast extract, and 1% swollen chitin. Every day, plates were checked for the presence of living chitinolytic fungi after being incubated at 30℃. Pure cultures of the isolates generating the largest clearing zones were selected. The diameter of the clearing zone divided by the colony's diameter yields the activity ratio.

Degradation of shrimp shell wastes

After being separated from the culture, the fungal strains were kept at -80°C in a preservation tube containing 25% glycerol. 100 mg of prepared shell waste were added to 250 mL flasks along with 100 mL of tap water. The broth made from shell debris has its pH corrected to 7.0. A 10% v/v inoculum of an overnight fungal culture cultured in 3% shell waste broth was added to each flask after sterilization, and the flasks were then incubated at 37°C on a rotary shaker (200 rpm/min). Remaining material cleaned and dried at 50°C for additional analysis at predetermined intervals. According to Iryna *et al*. (2009), degradation experiments were carried out in duplicate.

Determination of chitin decomposition (weight loss) with intact cells

According to Corroad and Tom (1978), the traditional liquid culture medium has the following contents in grams per milliliter: (NH4)2 SO4, 1.0; MgSO4.7H2), 0.3; KH2PO4, 1.36; and yeast extract, 0.5." The 250 ml ERLNMEYER flaks were filled with 50 aliquots. There was only one carbon source per flask: 1% chitinous solids, which are broken-down shrimp shell debris. The chosen fungi were added to the sterilized flasks, which were then cultured for a week at 30 degrees Celsius on a rotating shaker. After being incubated, any precipitates that were left over were lysed entire cells for one hour using 10% KOH. The lysates were then cleaned, filtered, and dried to calculate the amount of chitin that had been lost.

Separation of chitinase enzyme

For 48 hours, the *Aspergillus fumigatus* cultured in shaking flasks using the previously described standard liquid medium supplemented with 2% of decomposed shrimp shell debris. The medium was placed in a shaking water bath at 40 °C for two hours, and the supernatant was used to agitate 1% swollen chitin in citrate-PO_4 buffer (pH 6.6) using a centrifuge set at 10,000 rpm for 15 minutes at 4 °C (Paul *et al*., 2014). To inactivate the chitinase, the tubes were incubated for 3 minutes at 100 °C de a water bath. The solutions were centrifuged at 10,000 rpm for 15 minutes at 4 °C. The Paul *et al*., 2014 technique was used to test N-acetylglocoasamine (NAGA). Every therapy was administered three times.

Biochemical analysis of degraded shrimp shell waste

Lowry's (1951) method was used to determine the protein content of the shrimp shell deteriorated waste, and the colorimetric approach (Dubois *et al*., 1956) was used to assess the carbohydrates. The (Folsch *et al*. 1957) method was utilized to extract the total lipids from the dry shrimp shell degraded materials. An automatic amino acid analyzer (L-8900; Hitachi, Tokyo, Japan) was used to identify and quantify amino acids (Qin *et al*., 2021).Each sample was collected in triplicate, and the concentrations were expressed as a weight percentage of the decomposed shrimp shell waste.

GC-MS analysis

An integrated GC-QP 2015 (SHIMADZU) and mass spectrophotometer was utilized for the GC-MS analysis. It was equipped with an HP-5 MS fused silica column (5% phenyl methyl siloxane, 30.0 m × 250 μm, film thickness 0.25 μm), which was interfaced with a 5675C Inert MSD featuring a Triple-Axis detector. As the carrier gas, helium gas was adjusted to a column velocity flow rate of 1.0 milliliters per minute. Additional GC-MS parameters include: 250 °C for the ion-source; 300 °C for the interface; 16.2 psi for pressure; 1.8 mm for outtime; and 1 μl injector in split mode with split ratio of 1:50 and injection temperature of 300 °C. After five minutes at 36 °C, the temperature in the column increased to 150 °V at a rate of 4 °C per minute. At a rate of 20 °C per minute, the temperature was increased to 250 °C and maintained there for five minutes. Elution took 47.5 minutes in total. By comparing the average peak area of each component to the total areas, the relative percent amount of each component was determined. The system was controlled and the data was gathered using MS solution software, which was supplied by the supplier (Olivia *et al*., 2021).

FT-IR measurements and spectral collection

For FT-IR measurement, a thin translucent sample disc was formed by dispersing and encasing 10 milligrams of each freeze-dried shrimp shell degradation extract in 100 milliliters of potassium bromide (KBr). The disc was then put inside a diffuse reflectance accessory sample cup. To determine the distinctive functional groups in the sample, FT-IR studies were conducted using a PerkinElmer 2000 infrared spectrometer (Thermo Nicolet NEXUS 670, USA). The spectra were collected at a resolution of 4 cm^{-1} and within the range of 4000–400 cm^{-1} using Spectrum 10.03.09.0139 software® (PerkinElmer Inc., MA, USA) (Lina *et al*., 2019).

RESULTS

Fungi isolation and identification

Two distinct soil samples from the Mallipattinam region of east and west on Tamilnadu's southeast coast yielded a total of twenty-five isolates when analyzed. From agar plates that were cultured for seven days at 28°C, six strains were identified. By transferring a single conidial onto beer agar plates, all fungal isolates were recovered in pure cultures (**Table 1**). *Aspergillus fumigatus*, *A. terreus*, *A. niger*, *A. flavus*, *Pencillium citrinum*, and *Trichoderma viride* were the names of the fungal isolates (**Figure 1**).

Table 1: Estimation of chitinase enzyme activity of potential fungi from degraded shrimp shell waste

Name of the fungi	Zone of clearance (µg moles/ml/min)	Degradation of shrimp shell (-) Absent (+) present
Aspergillus flavus	0.12±0.20	-
A. fumigates	0.67±0.23	+
A. niger	0.19±0.21	-
A. terreus	0.45±0.26	-
Penicillium citrinum	0.23±0.18	-
Trichoderma viride	0.53±0.35	-

Determination of weight loss

The fungi were cultivated using 1% deteriorated shrimp shell debris in order to facilitate hydrolysis with intact cells. **Figure 1** displays the percentage weight loss of the chitin substrates in the culture flasks. *Aspergillus fumigatus* was found to have the highest effect on weight loss when combined with colloidal chitin, whereas shrimp shell waste showed the best effects.

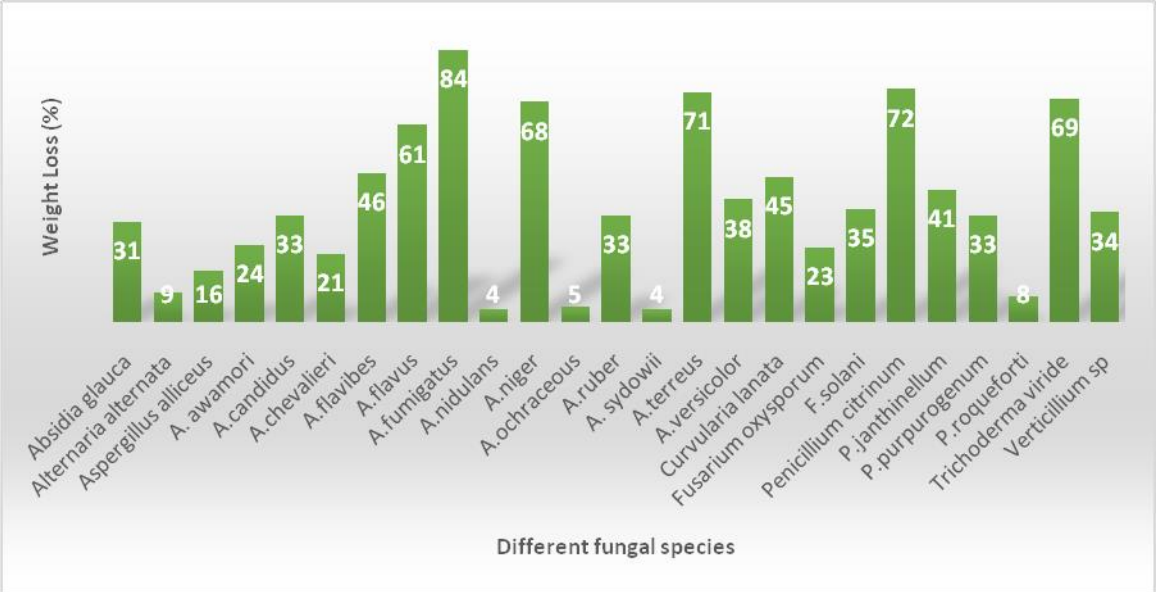

Figure 1: Percentage weight loss of degrading shrimp shell waste by fungi

Biochemical analysis of degraded shrimp shell waste

The biochemical compounds were analyzed from shrimp shell waste. The degraded samples contained trash that was primarily made up of protein (23.5%) and chitin (25.3%). When compared to the untreated shrimp waste samples, the largest amount of fatty acids were discovered in the degraded shrimp samples (**Figure 2**)

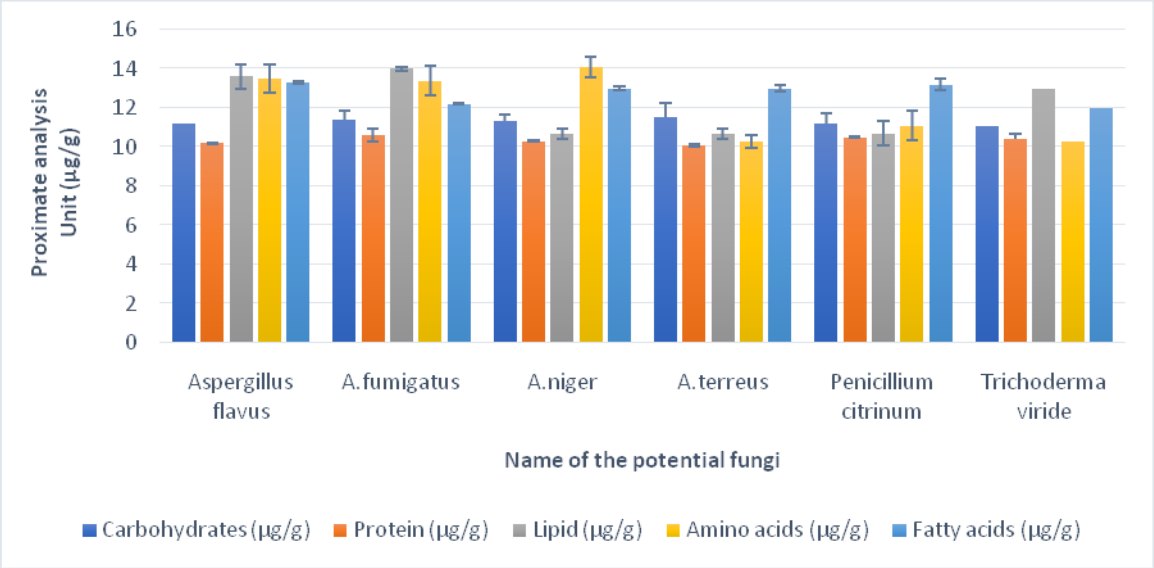

Figure 2: Chemical composition of degraded shrimp-shell waste

GCMS analysis

The presence of 15 bioactive chemicals was shown by the examination results of the deteriorated shrimp shell waste (**Figures 3**). The phytochemical compounds were identified using the molecular formula, peak area, and retention time. The compound name, probability, area percent, retention duration, and chemical formula were displayed in **Table 2**. Degraded shrimp shell waste analysis revealed that 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester was detected as a major compound by (14.5%), followed by Ethanol, 2-Butoxy (4.14%), Cyclohexasiloxane, dodecamethyl (11.66%), Cyclohexanone, 2-Chloro-3-(1,1-Dimethylethyl)-, Trans-(.+-.) (4.61%), 4-(Dimethylamino) Azoestrone 3-Methyl Ether (2.27%), (S)-1,1-Dimethylethyl 3-Hydroxy-2-Methyl-5-Phenyl-4-Pentenedithioate (7.1%), Cesium Trimethylfluoro) Aluminate (4.92%), Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester (9.71%), Tri-O-Trimethylsilyl, N-Pentafluoropropionyl derivative of terbutaline (11.8%), Tri-O-trimethylsilyl, N-heptafluorobutyryl derivative of terbutaline (9.15%), Tri-O-trimethylsilyl, N-pentafluoropropionyl derivative of terbutaline (5.1%), 12-Azabicyclo(9.2.1)tetradeca-1(14)-ene-13-one (2.73%), 4-(Dimethylamino) azoestrone 3-methyl ether (6.16%), 3,4-dihydroxyphenylglycol, 4TMS derivative (4.17%), 1H-indole-3-carboxylic acid, 2-PHENYL-1-(phenylmethyl)-, ethyl ester (1.94%).

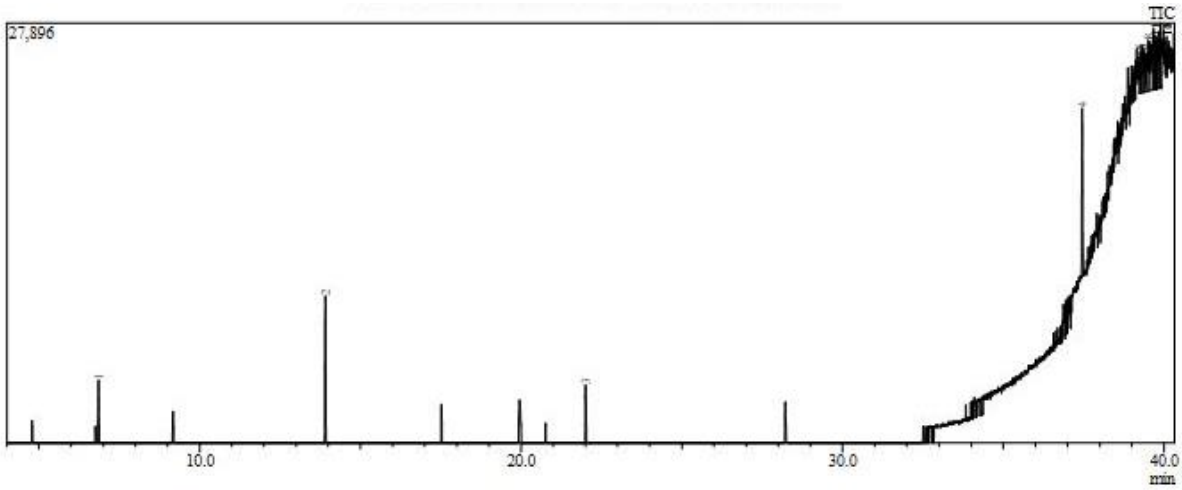

Figure 3: Chemical compounds of shrimp shell waste after treatment by *A. fumigatus* analyzed by GCMS method

Table 2: Analysis of chemical components shrimp shell degraded with *A. fumigatus* by GCMS method.

Retention time	Name of the compounds	Area (%)	Molecular formula	Molecular weight (kDa)
6.85	Ethanol, 2-Butoxy-	4.14	C_4H_8O	72
13.909	Cyclohexasiloxane, dodecamethyl-	11.66	$C_{12}H_{36}O_6Si_6$	444
22.005	Cyclohexanone, 2-Chloro-3-(1,1-Dimethylethyl)-, Trans-(.+-.)-	4.61	$C_{10}H_{17}C_{lO}$	188
37.454	1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	14.54	$C_{24}H_{38}O_4$	390
39.258	4-(Dimethylamino) Azoestrone 3-Methyl Ether	2.27	$C_{24}H_{38}O_4$	398
39.34	(S)-1,1-Dimethylethyl 3-Hydroxy-2-Methyl-5-Phenyl-4-Pentenedithioate	7.1	$C_{17}H_{26}OS$	278
39.4	CesiumTrimethylfluoro) Aluminate	4.92	$C_8H_{17}N_2O_3P$	220
39.515	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester	9.71	$C_{16}H_{18}N_4O_3$	314
39.615	Tri-O-Trimethylsilyl, N-Pentafluoropropionyl derivative of terbutaline	11.8	$C_{25}H_{42}F_7NO_4Si_3$	637
39.665	Tri-O-trimethylsilyl, N-heptafluorobutyryl derivative of terbutaline	9.15	$C_{24}H_{42}F_5NO_4Si_3$	587
39.735	Tri-O-trimethylsilyl, N-pentafluoropropionyl derivative of terbutaline	5.1	$C_{25}H_{42}F_7NO_4Si_3$	637
39.784	12-Azabicyclo(9.2.1)tetradeca-1(14)-ene-13-one	2.73	$C_{14}H_{24}O_2$	224
39.843	4-(Dimethylamino)azoestrone 3-methyl ether	6.16	$C_{19}H_{39}NO_3Si_3$	413
39.89	3,4-dihydroxyphenylglycol, 4TMS derivative	4.17	$C_{20}H_{42}O_4Si_4$	458
40.081	1H-indole-3-carboxylic acid, 2-PHENYL-1-(phenylmethyl)-, ethyl ester	1.94	$C_{21}H_{29}N_3O_2$	355

FTIR analysis

Figure 4 displays the FT-IR spectra of deteriorated shrimp shell debris. Numerous absorption peaks with centers at 3322.07, 2941.51, 2832.25, and 1901.01 cm−1 were clearly visible in the spectra. The absorption peak, centered at 2525.87 cm−1, was associated with numerous bands of vibration, hydroxyl stretching (bounded), carboxylic acids, and maybe ester bonds. The stretching of the carboxylate anion was correlated with the wave number of 1421.65 cm−1. The strong signal at 1112.06 cm−1 was associated with secondary alcohols, O-H bounding and C-O stretching vibrations, which were produced by the protein's carbonyl stretch bond vibrations.

DISCUSSION

The goal of fungal taxonomy is to identify, categorize, and characterize every species of fungus while also provide instruments for doing so. About 100,000 fungal species have been described as a consequence of specimen-based research. It will take centuries or millennia to characterize every fungal species on Earth before they become extinct, based on the regression relationship between the number of fungal species described and years. Therefore, it is imperative to accelerate the speed of species description in order to approach a comprehensive library of fungal diversity within a tolerable time frame. However, it is extremely doubtful that the goal will be accomplished in the near future due to the drawbacks of conventional morphology-based taxonomy and the large number of active taxonomists (Purvis and Hector A. 2000). In this study, two distinct soil samples from Mallipattinam, on southeast coast at Tamilnadu, yielded a total of twenty-five fungus strains. *Aspergillus alliaceus, Absidia glauca, Alternaria alternata, Aspergillus fumigatus, A. nidulans, A. niger, A. ochraceous, A.*

ruber, *A. sydowii*, *A. terreus*, *A. versicolor*, *Penicillium citrinum*, *P. janthinellum*, *P. purpurogenum*, *P. roqueforti*, *Trichoderma viride*, and *Verticillium* sp. were among the soil fungi isolated and identified in the current study. It is well known that a large number of microorganisms that are essential to the soil ecology store their energy in the soil (Stefanis *et al*., 2013).

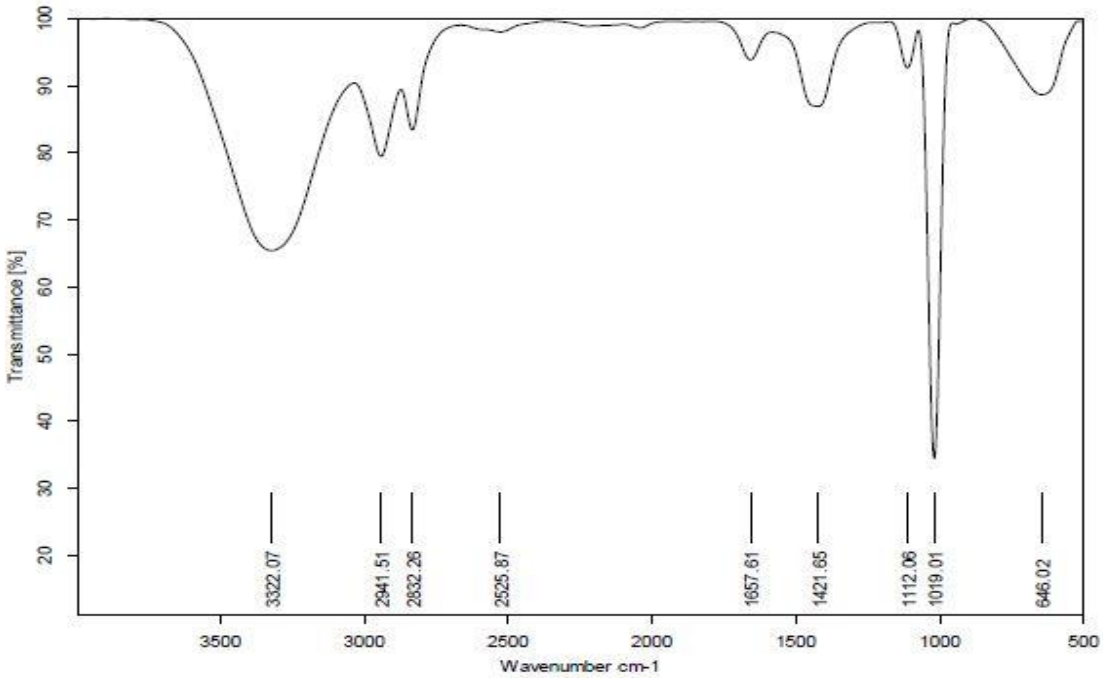

Figure 4: FT-IR analysis of functional groups of shrimp shell waste degraded by *A. fumigatus*

Aspergillus was the most commonly recognized genera, and it was isolated from all of the soil samples under study. In reclaimed soil from shrimp waste deposited soil, *Aspergillus* sp., *A. niger*, and *A. tubingensis* were more prevalent in Mallipattinam, on southeast coast at Tamilnadu's. While *A. ochraceus* and *A. chevaliers* were only discovered in the control soil, which has never been cultivated, *A. flavus*, *A. aculeatus*, and *A. fumigatus* were found in both the reclaimed and the uncultivated samples.It was discovered that *Aspergillus terreus* was the only one connected to the marine waste environment. *Fusarium* was found to be the second most often isolated genus. Only the decomposed soil included *F. solani* and *F. acuminatum*, but *F. oxysporum* was isolated from every soil sample examined. On the other hand, *F. brachygibbosum* was discovered to be connected to the marine contaminated soil, while *F.equiseti* was only isolated from soil that had been contaminated by discarded shrimp excrement. *P. digitatum* was identified from soil that had been contaminated by discarded shrimp waste, *P. oxalicum* and *P. citrinum* were isolated from samples of reclaimed land, and Genera *Penicillium* was also isolated from all soil samples. From both samples, isolates of *Trichoderma viridie* and *T. harzianum* were obtained. *Curvularia lunata* was only found in the soil that had been deposited with shrimp feces. The findings were consistent with those of other investigations (Hossain and Iqbal, 2014; Nouri *et al*., 2016; Krishnaveni and Ragunathan, 2014) that identified the same genera of fungi.

Fungal volatile metabolites of several chemical classes were formed during the *Aspergillus* fungus' breakdown of prawn shell waste for the purpose of chitin extraction. The culture supernatant contained fatty acid derivatives, including hydrocarbons, ketones, alcohols, organic acids, and substances containing sulfur and nitrogen. The proteins, lipids, and minerals that remained in the prawn shell waste were broken down by various catabolic processes, including glycolysis, proteolysis, and lipolysis, to yield these metabolites (Jenin *et al*., 2016). The identified linear-chained hydrocarbons were most likely originated from fatty acid biosynthesis pathway products.The culture supernatant included both longer-

chain hydrocarbons and short-chain alkanes, indicating that the microbial strains were capable of synthesizing branched hydrocarbons. This study examines the biochemical makeup of degraded shrimp feces. When compared to shrimp waste, the degraded waste had the highest protein content. The majority of the fungi were efficient biodegraders. According to Suresh and Anil Kumar (2012), it's possible that fungi break down the debris from shrimp shells, converting macromolecules like glucose and amino acids into smaller ones.

For the generation of chitinase and the breakdown of shrimp waste, the effects of three different parameters were estimated. Table 2 shows that the degradation of shrimp waste ranged from 12% to 52%, and the enzyme production varied from 2.45 IUml-1 to 4.21 IUml-1. A Pareto chart (Fig. 2) illustrates the effects of several parameters on the breakdown of shrimp waste and the formation of chitinase. Among all of these fungal species, there was a significant favorable impact on the generation of chitinase and the breakdown of shrimp waste. The breakdown of shrimp shell debris by fungal strains has been documented in literature thus far.

Chitinase production is impacted by the presence of trace elements, such as minerals, in the production medium. It is commonly known that Mg2+ ions play a critical role in the stability and production of cell growth enzymes. Rhizopus sp. AD's increased chitinase yield is consistent with Ullah *et al*. (2017) findings that KCl and MgSO4 promote chitinase synthesis in Humicolagrisea and Bacillus spp., respectively. A different study found that when exposed to MgSO4 concentrations, Serratia marcescens can manufacture more chitinase (Brurberg *et al*., 2000). A study that looks at and optimizes a number of criteria for the disintegration of shrimp waste is not readily available.

Table 3 provides a summary of the different bonds found in chitin along with the spectral bands that correspond to them. The *Aspergillus* species-treated deteriorated shrimp wastes' FTIR spectra. According to Durate *et al*. (2001) and Ravindra *et al*. (1998), degraded shrimp wastes displayed peaks at 2941.51 cm-1, 1621 cm-1, 1657.61 cm-1, and 1112.06 cm-1 that were indicative of chitin. Due to aromatic skeleton vibrations inside plane deformation, the peak size at 645.02 cm-1, which corresponds to the –OCH3 group in the case of the untreated sample, increased coupled with a band shift to 1432 cm1.The intensity of the other peaks likewise changed. These alterations all point to chitinaceous substance deterioration. However, following enzymatic treatment, the hydrolysate's FTIR spectra showed a significant increase in the peak size at 3322.07cm-1. It was explained by the creation of -OH groups and the breaking of links in the chitin chain. These alterations all point to the production of chitin oligosaccharides, which are byproducts of chitin hydrolysis. The chromatograms of the samples taken from the shrimp shell waste and after *A. fumigatus* degradation are shown in Figs. 3 and 4, respectively, in the current investigation.

Table 3: Analysis of functional group of chemical compounds degraded with *A. fumigatus* by FT-IR method

Group frequency cm^{-1} of the sample	Functional group assignment
3322.07	Amines, Imines (=N-H); one hand
2941.51	Hydrocarbon chromophore, C-H Stretching, Alkane
2832.25	Aldehydes C-H Stretching vibration
2525.87	Carboxylic acids, Hydroxyl stretching (bounded), several bands
1657.61	Amides, secondary solid and concentrated solution
1421.65	Carboxylate anion stretching
1112.06	O-H bounding and C-O stretching vibrations, secondary alcohols
1019.01	O-H bounding and C-O stretching vibrations, primary alcohols
645.02	Sulfor compounds, sulfonic acid

Tables 2 and 3 list the discovered compounds along with their retention time, molecular weight, molecular formula, peak area (%), and activity. Twelve pyrazines, eight hydrocarbons, twelve ketones, one N-containing molecule, seven alcohols, four aldehydes, one heterocyclic compound, two esters, and one S-containing compound were among the fifteen bioactive compounds found in the degraded shrimp waste that were found and measured (Table 2). Fifteen chemicals with diverse biological properties were found in the degraded shrimp shell waste from *A. fumigatus*, according to the results of the GCMS study.

The major compounds identified were 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (14.54%), followed by Tri-O-Trimethylsilyl, N-Pentafluoropropionyl derivative of terbutaline (11.8%), cyclohexasiloxane, dodecamethyl- (11.66%), Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester (9.71%), and Tri-O-trimethylsilyl, N-heptafluorobutyryl derivative of terbutaline (9.15%). The minor compounds analyzed were 1H-indole-3-carboxylic acid, 2-PHENYL-1-(phenylmethyl)-, ethyl ester (1.94%), 12-Azabicyclo(9.2.1)tetradeca-1(14)-ene-13-one (2.73%), and 4-(Dimethylamino) Azoestrone 3-Methyl Ether (2.27%). The entire degraded shrimp shell waste contained 15 volatile chemicals. The volatile compounds' compositions and contents vary depending on the component. They made up over 80% of the total and possessed a large portion. Because of their large quantity and low threshold, pyrazine and pyridine odorants—which have been found in shrimp products before—are regarded as an important class of odorants that resemble cooked and roasted meat (Mall & Schieberle, 2016; Zhang *et al*., 2022). The Maillard reaction and interactions with results of lipid degradation are the primary processes that create these chemicals (Wheatley 2002). The waste from freshwater prawn shrimp shells differed noticeably, with very little pyrazine (less than 1% of the total). The findings showed that the chitin from shrimp shells included a large number of precursors to fragrance chemicals.

CONCLUSION

When shrimp shell waste is used to break down *Aspergillus fumigatus*, many beneficial compounds are created. In addition to the enzyme chitinase, the culture supernatant contained a number of organic acids, including fatty and amino acids, as well as various antimicrobial compounds. This demonstrates that proteins, minerals, and lipids are successfully extracted from the shrimp shells using chitinase-mediated protein hydrolysis. The nutrient-rich liquid portion of the breakdown may be utilized for therapeutic purposes, while the solid portion is harvested and converted into chitin.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the services rendered by the management and Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur for the successful completion of Research work.

REFERENCES

Arbia, W., Adour, L., Amrane, A., *et al.,* 2013. Optimization of medium composition for enhanced chitin extraction from Parapenaeus longirostris by Lactobacillus helveticus using response surface methodology. *Food Hydrocolloids*, **31**:392- 403.

Barron, G.L. 1968. The genera of hypomycetes from soil, the Williams and Wilkins Co. 2. Balttimore, pp.364.

Brurberg, M.B., Synstad, B., Klemsdal, S.S., *et al.,* 2000. Chitinase from Serratia marcescens. *Microbiology*, **4**:212-218.

Chen, L., Wei, Y., Shi, M., *et al.,* 2019. An Archaeal Chitinase with a Secondary Capacity for Catalyzing Cellulose and Its Biotechnological Applications in Shell and Straw Degradation. *Frontiers in Microbiology*, **10**:1253

Das, M., Ghosh, S., Dash, B., *et al.,* 2013. Multifarious utilization of shrimp waste at Visakhapatanam, Marine Fisheries Information Service T&E Ser., 901 No. 216.

Domsche, K.H. and Gams, W. 1972. Fungi in agricultural soils. Longman Group Ltd., London, pp.290.

Dubois, M., Gilles, KA., Hamilton, JK., Rebers ,PA., and Smith, F. 1956. Calorimetric method for

determination of sugars and related substances. *Analytical Chemistry*, **28**:350-356.

Dutta, P.K., Ravikumar, M.N.V., and Dutta, J., 2002. Chitin and chitosan for versatile applications. *Journal of Macromolecular Science Polymer Reviews*, **42**:307-354.

Ellis, M.B. 1976. More domatiaceous hyphomycetes. Common Wealth Mycological Institute. Kew, London, pp.608.

Folch, J.M.L., Lees, M.P. and Stanely, S.G.H.A. 1957. A simple method for the isolation of total lipids from animal tissues. *Journal of Biological Chemistry*, **226(1)**:497-509.

Food and Agriculture Organization of the United Nations, 2020. The state of World Fisheries and Agriculture, Food and Agricultural Organization of the United Nations.

Hossain, M.S. and Iqbal, A. 2014. Production and characterization of chitosan from shrimp waste. *Journal of Bangladesh Agricultural University*, **12**:153-160

Sorokulova, I., Krumnow, A., Globa, L., *et al.*, 2009. Efficient decomposition of shrimp shell waste using *Bacillus cereus* and *Exiguobacterium acetylicum*. *Journal of Industrial Microbiology and Biotechnology*, **36**:1123-1126.

Jenin, G.A., Babu, M.M., Murugan, M., *et al.*, 2016. Isolation and Identification of Chitinase Producing Native Fungi From Saltpan of Puthalam, Kanyakumari District, Tamil Nadu, India. *Journal of Applied Biology and Biotechnololgy*, **4**:001-005

Kreag, R. and Smith, F. 1975. Seafood solid waste in Oregon: disposal or recovery? Oregon State University Extension Marine Advisory Program Report, pp.395.

Krishnaveni, B. and Ragunathan, R. 2014. Chitinase from marine wastes by *Aspergillus terreus* and its application in degradation studies. *International Journal of Current Microbiology and Applied Sciences*, **3**:76-82.

Lowry, O.H., Rose Browgh, N.J., Farr, A.L., *et al.*, 1951 Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, **193**:265-275.

Faisal, M., Elhussieny, A., Ali, K.A., *et al.*, 2017. Extraction of degradable biopolymer materials from shrimp shell wastes by two different methods. IOP Conference Series: Materials Science and Engineering. The 4th International Conference on Advanced Sciences, Hurghada, Egypt, **464**:7- 10.

Mall, V. and Schieberle, P. 2016. Characterization of Key Aroma Compounds in Raw and Thermally Processed Prawns and Thermally Processed Lobsters by Application of Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*, **64(33)**:6433-42.

Nouri, M., Khodaiyan, F., Razavi, S.H., *et al.*, 2016. Improvement of chitosan production from Persian Gulf shrimp waste by response surface methodology. *Food Hydrocolloids*, **59**:50-58.

Olivia, N.U., Goodness, U.C. and Obinna, O.M. 2021. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction. *Future Journal of Pharmaceutical Sciences*, **7**:59.

Paul, S., Jayan, A., Sasikumar, C.S., *et al.*, 2021. Extraction and Purification of Chitosan from Chitin Isolated from Sea Prawn (Fenneropenaeus Indicus). *Asian Journal of Pharmaceutical and Clinical Research*, **7**:201-4.

Purvis, A. and Hector, A. 2000. Getting the measure of biodiversity. *Nature*, **405**:212-219.

Qin, Y., Li, X., Li, J., *et al.*, 2021. Seasonal variations in biochemical composition and nutritional quality of Crassostrea hongkongensis, in relation to the gametogenic cycle. *Food Chemistry*, **356**:129736.

Sachindra, N.M., Bhaskar, N. and Mahendrakar, N.S., 2005. Carotenoids in different components of Indian Shrimps. *Journal of the Science of Food and Agriculture*, **85**:167-172.

Stefanis, C., Alexopoulos, A., Voidarou, C., *et al.*, 2013. Principal methods for isolation and identification of soil microbial communities. *Folia Microbiology* (Praha), **58(1)**:61-68.

Suresh, P.V. and Anilkumar, P.K. 2012. Enhanced degradation of α- chitin materials prepared from shrimp processing by product and production of N- acetyl -D glucosamine by thermo active chitinases from soil measophilic fungi. *Biodegradation*, **23**:597-607.

Ullah, I., Khan, N.A., Jadoon, M.A., *et al.,* 2017. Anwar S. Isolation and identification of different rhizospheres fungi of Mansehra region, Pakistan. *Journal of Entomology and Zoology Studies*, **5(2)**:437-442.

Wheatley, R.E. 2002. The consequences of volatile organic compound mediated bacterial and fungal interactions. *Antonie Van Leeuwenhoek*, **81**:357-364.

Wu, H., Ge, M., Chen, H., *et al.,* 2020. Comparison between the nutritional qualities of wild- caught and rice-field male Chinese mitten crabs (Eriocheirsinensis). LWT, 117:108663.

Xu, Y., Bajaj, M., Schneider, R., *et al.,* 2013. Transformation of the matrix structure of shrimp shells during bacterial deproteination and demineralization. *Microbial Cell Factories*, **12**:90.

Xu, Y., Gallert, C., and Winter, J., 2008. Chitin purification from shrimp wastes by microbial deproteination and decalcification. *Applied Microbiology and Biotechnology*, **79**:687-697.

Yang, L., Zhang, A., and Zheng, X., 2009. Shrimp Shell Catalyst for Biodiesel Production. *Energy Fuels*, **23**: 3859-3865.

Zhang, Z., Ji, H., Zhang, D., *et al.,* 2022. The Role of Amino Acids in the Formation of Aroma-Active Compounds during Shrimp Hot Air Drying by GC-MS and GC-IMS. *Foods*, **11(20)**:3264.