WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 2080-2096. Research Article ISSN 2277– 7105

ENZYMATIC AND ANTIMICROBIAL ACTIVITIES OF *FUSARIUM* ASSOCIATED WITH *CURCUMA LONGA* AND THEIR POSSIBLE APPROACHES IN VARIOUS INDUSTRIES

Smruti Mahapatra[1]*, Sweta Mishra[1], Subhasrita Mishra[1] and Dr. V. Suneetha[1]

Department of Biotechnology, School of Bioscience and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu, India.

Article Received on
12 May 2020,

Revised on 01 June 2020,
Accepted on 21 June 2020,

DOI: 10.20959/wjpr20207-17961

***Corresponding Author**
Smruti Mahapatra
Department of Biotechnology, School of Bioscience and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu, India.

ABSTRACT

A member from the family of ginger (Zingiberaceae), *Curcuma longa* (Turmeric) is known in every household since ages due to its uses covering fields like medicine, food, dye, cosmetics and its uses in various spiritual and cultural practices. There have been many proven antimicrobial properties of turmeric. Contradictorily it is also seen to harbor certain endophytic fungi that can synthesize various secondary metabolites. *Fusarium* species have been found predominantly colonizing on *C. longa*. This genus notably affects the rhizomes of various vegetable plants, includes other plant pathogens, and also produces various mycotoxins. In some cases it has also been reported to control insects with promising anti-microbial and pest control characters like abundant sporulation and fast action causing a high mortality rate in plant pathogens. These phytopathogenic fungi can efficiently produce very specific extracellular enzymes that have and can have potential and practical applications in various bio-conversions, in the agro-industry and various biotechnological processes. This study endeavors to exploit various enzymes and secondary metabolites produced by the species and look for certain economical industrial applications of these enzymes. Turmeric infected with the fungus was used as a source for isolating *Fusarium* and purifying it through streaking and sub culturing. Its antimicrobial properties was checked through well diffusion method alongside checking its effect on adventitious roots like potato, colocassia, onion, ginger, carrot and tomato as control. Screening for various applications of various enzymes produced by *Fusarium* was also checked for.

KEYWORDS: *Fusarium, Curcuma longa,* phytopathogenic fungi, Extracellular enzymes, Antimicrobial activity, Amylase.

INTRODUCTION

Fusarium spp. has been denoted as mycotoxin producers as well as major plant Pathogenic fungi. Apart from that they are also known for endosymbiotic nature that is it is an endophyte, That ubiquitously exists in plants for the most part without even causing any harm or disease rather acting as a source of bioactive substances that might be helpful for the plant.[1] These species of fungus are known to produce bioactive substances which potentially act against virus, diabetes, cancer, or mostly against bacteria.[2] In some cases these fungi have also show the efficiency of control of pests and insects in crop plants through processes like abundant sporulation and fast action causing a high mortality rate in plant pathogens.[2] But surprisingly it is seen to infect *Curcuma longa* which is an ancient coloring spice used predominantly in Asian countries. In recent years curcumin is very trendy in scientific researches due to some of its special and unique characteristics.[3] The content of curcumin in a *Curcuma longa* plant varies like many other plant varieties based on various factors like geographical region, hybridization with other variety of *Curcuma longa* species. So for a significant affect a plant that has a higher content of curcumin should be used for research purposes.[4] It is seen that curcuminoides, which are known as some photochemical compounds, are found in *Curcuma longa*. *Curcuma longa's* rhizome is commonly known as turmeric.[5] For textile material, turmeric can also act as an antimicrobial agent. By the exhaustion method Curcumin along with chitosan and aloevera act against microbial growth in rabbit hair assessed, cotton, wool, etc.[6] Various studies show that it can possess various effects such as anti-inflammatory, anti-gastroprotective activities, antioxidant, along with antimicrobial, anti-tumor activities.[3] Raw or dried turmeric rhizome which is the rhizome of *Curcuma longa* also has insect repellence as well as antimicrobial activities and used in traditional medicine practice.[7][8] Hexane and methanol combination extracts of *C. longa* has some antibacterial effect which is against around 13 bacteria of different genera like *Vibrio, Bacillus, Aeromonas, Streptococcus, Staphylococcus namely Vibrio harveyi, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Bacillus subtilis, Bacillus cereus, Aeromonas hydrophila, Streptococcus agalactiae, Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus epidermidis, and Edwardsiella tarda. however, curcuminoids can inhibit eight bacteria of Streptococcus agalactiae, Staphylococcus intermedius, Staphylococcus epidermidis, Staphylococcus aureus, Aeromonas hydrophila,*

Bacillus subtilis, Bacillus cereus, and Edwardsiella tarda.[9] Turmeric oil derieved during curcumin manufacture has shown inhibitory effect against *Bacillus coagulans, Bacillus subtilis, Bacillus cereus, Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*[10] Curcumin also has shown certain anti-biofilm activity against *P. aeruginosa* strains of two types.[11] Curcumin is a potential reductant of biofilm initiation genes, which inhibit 31 quorum sensing (QS) genes; It also causes down-regulation of acyl-homoserine lactone (HSL) production, which is a virulence factor, elastase/protease activity, and also cause biosynthesis of pyocyanin.[7] The emergence of antiviral drug resistance, loss of powerful viral retailers, and the high fee of a few antiviral treatments boost up research to discover new effective antiviral compounds.[12][13] it's been demonstrated that curcumin as a plant by-product has a wide variety of antiviral pastime towards distinct viruses. in the de novo synthesis of guanine nucleotides Inosine, monophosphate dehydrogenase (IMPDH) enzyme because of its price-proscribing activity act as a therapeutic goal for antiviral and anticancer compounds.[14] Curcumin has such a lot of derivatives, which can be called reduced curcumin, allyl-curcumin, and tocopheryl-curcumin.[15] The turmeric act as a considerable inhibitor against fungal contaminations whilst delivered to plant tissue subculture on the zero. eight and 1.0 g/L.[16] Turmeric is not only used word wide as one of the most needed spices in regular food and a must-have in the kitchen but also it is used in the pharmaceutical industry and for medicinal purposes along with its use as a dying agent and in some cases, it is used as preservative like in preserving fish.[17] Despite its different proved antifungal and antibacterial properties the surface of the *C. longa* has seen to harbor certain phytopathogenic or endophytic fungus. The most common are the *Fusarium* species that have been identified till now. *Fusarium* species is a very economically and scientifically important stain of fungus known to produces not only mycotoxins and cause diseases but for the production of various extracellular secondary metabolites or enzymes that have different effective applications in various industries.[18] Enzymes are a key ingredient in various industrial sectors including textile, pharmaceutical, food as well as chemical sectors, and are very helpful in different bio-conversion processes.[19] In this study, some light was showed up on the different antimicrobial and enzymatic activity of fungus, that is *Fusarium* species, and its effect on various adventitious roots like potato, colocasia, onion, ginger, carrot, and tomato was seen and its dominance over *Aspergillus* species was also checked by inoculating the *Fusarium* from *C. longa* and preparing pure cultures.the isolate was also screened for different enzymatic activities like amylases, proteases, and cellulases.

MATERIAL AND METHODS

Sample collection

The infected rhizomes of the *Curcuma longa* consisting of white cottony colonies were used as the sample. The sample was collected from the vegetable market of Bhubaneswar, Odisha, India.

Fig. 1: Infected turmeric rhizome with white fungal colonies.

Isolation of the fungal species

For the isolation of the required fungus, firs the *Curcuma longa* rhizomes were first surface sterilized. This was done by first washing the rhizomes using 70% ethanol for three minutes, then with 5% NaOCl for three minutes again with 70% ethanol for thirty seconds. The rhizomes are then washed with distilled water three to four times.[19] After the sterilization process, the rhizomes were cut into thin slices using a sterilized scalpel. The slices were placed on sterilized Petri plates with Czapekdox agar and PDA aseptically using sterilized forceps. Then the plates were kept at 25-degree centigrade for 3-4 days.

Fig. 2: Sterilized sliced turmeric rhizome.

Microscopic examination of isolated fungi

After the incubation identity of the fungal genus was confirmed by performing lacto phenol cotton blue (LPCB) mounting and the stained slides were viewed under microscope for analysing their morphological characteristics. It was seen that the growth of fungal culture was adequate in the Czapekdox broth medium.

Preparation of pure culture plates

Further single colonies from those plates were streaked in fresh Czapekdox agar plates as growth was better in Czapekdox agar media plates. The colonies those were taken for LPCB only were used. The streaked plates were then incubated for 3-4 days.

Preparation of seed culture

Then Czapekdox broth was prepared and sterilized. Single colonies from previously streaked Czapekdox agar plates were then inoculated in sterilized Czapekdox broth and were then incubated at 25ºC, after 3-5 days again LPCB mounting was performed.

Effect of fungal species on various root vegetables

A simple procedure was performed for studying how the *Fusarium* spp. infect different root vegetables. For this procedure potato (*Solanum tuberosum*), carrot (*Daucus carota*), colocasia (*Colocasia esculenta*), ginger (*Zingiber officinale*), onion (*Allium cepa*) were taken as samples and tomato (*Solanum lycopersicum*) was used as control. Each of these vegetables were kept as a whole in separated sterilised beakers and 1ml of seed culture was inoculated in each of these beaker and then the beakers were sealed by using aluminum foil to prevent any contamination these are kept for 5-7 days for proper incubation.

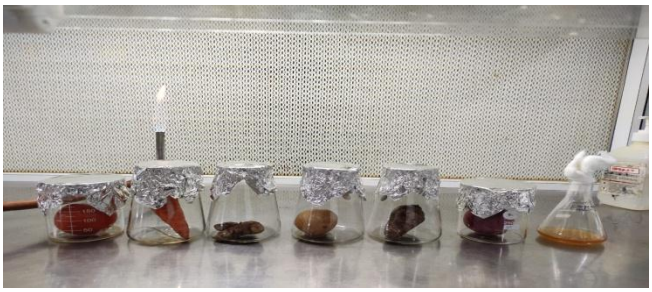

Fig. 3: Root vegetables with fungal inoculums.

Dominance check-up of fungal species

To check the dominance of *Fusarium* species on *Aspergillus* spp. on onion the onion sample was done by again inoculating it with 1-2ml of *Fusarium* seed culture and incubating for 5-7 days.

Antimicrobial test

For checking the antimicrobial activity of the isolated fungal spp. 1ml of seed culture was again inoculated in Czapekdox broth and incubated at 25ºC for 15 days, 2ml of this culture was centrifuged for 10 minutes at 4 ºC and at 6000rpm. The supernatant from the ependroff

tube was collected and this was used as sample for the antimicrobial test. The antimicrobial activity was checked against *Escherichia coli* and *Staphylococcus aureus*. Muller Hinton agar plates were prepared, sterilized and inoculated with a even bacterial lawn using cotton swab dipped in the overnight bacterial cultures of the given strain and left for a few minutes for drying and then wells were made using borer and well diffusion method was performed by adding about 50 µl of the sample, positive and negative control into the wells.[2] The antimicrobial activity test was performed by using streptomycin which was taken as positive control and distilled water which was taken as negative control. The result of this test was concluded by observing and measuring the halo zone of inhibition found around the sample and the positive control.

Enzymatic activity test

The enzymatic activity of the sample was also checked by performing various enzymatic assays; the assays are performed for enzymes like amylase, protease and cellulase. For the amylase, protease and cellulose activity starch casein agar, skimmed milk agar and modified Czapekdox-cellulose agar (4.9gm Czapekdox agar and 1gm of cellulose powder was taken in a sterilised beaker and 100ml of distilled water was added) was used.[18] The fungal seed culture was then streakedon sterilised agar plates and were incubated at 25 degree centigrade for 3 days for amylase and protease activity and 5 days for cellulase activity. After incubation the modified Czapekdox-cellulose agar plate and skimmed milk agar plates were taken and flooded with Congo red and grams iodine respectively and the activity of enzymes was confirmed by observing the halo zones around the colonies and the amylase activity can also be confirmed by observing the halo zones around the colonies.[18]

RESULTS

Fusarium infection is the measure cause of spoilage of root vegetables worldwide. *Fusarium* produces many bioactive compounds like enzymes. These enzymes are known to be natural and are not harmful like the artificial enzymes so these can be used as a alternative source of enzymes in various industries like food, detergent, leather industries, etc.

Isolation of fungal species

After incubating at 25ºC for 3-5 days adequate amount of fungal growth was seen on the petri plates. Therefore, to know what the identity of fungus grown on the plate is further test was carried out.

Fig. 4: Fungal growth on the Petri plates Containing the suitable media.

Fig. 5: Prepared seed culture.

Morphological Characterization of fungal spp

The morphological structures like sporangia, hyphae, mycelium resembles to the fungal genus *Fusarium*. According to various studies this fungal spp. is mainly responsible for the wilt disease of various root vegetables.

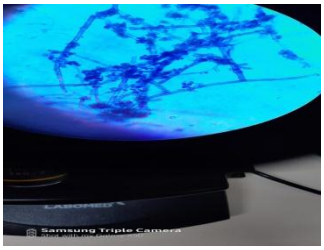

Fig. 6: LPCB mounting of fungal species.

Effect of fungal spp. on various root vegetables

After inoculation with 1 ml sample and proper incubation white colour growth was observed on various root vegetables and no growth was seen on the control.

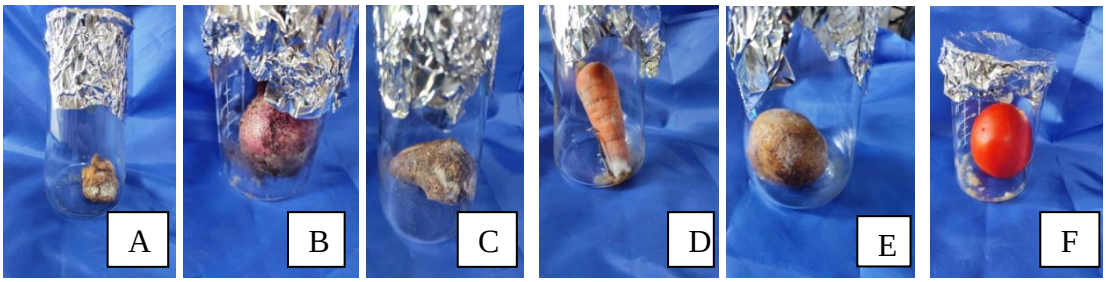

Fig. 7: Fungal growth on A- Ginger B- Onion C- Colocasia D- Carrot E- Potato F- No growth on tomato.

Dominance check-up test

On the onion sample growth of other fungus was seen as black colonies morphological characterization of the fungus was confirmed by performing LPCB mounting for the dominance check-up and the onion sample was again inoculated with 1ml of culture and then

it was observed that both the *Aspergillus* spp and *Fusarium* do not inhibit each other and rather grow together simultaneously.

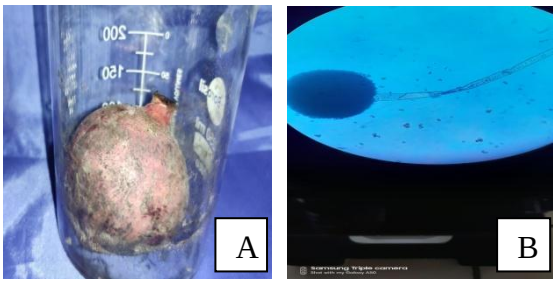

Fig. 8: A-Growth of both *Fusarium and Aspergillus* on onion B- LPCB mounting of Aspergillus.

Antimicrobial test

The presence of antimicrobial activity in the isolate was checked by making Muller Hinton agar plates and forming a bacterial lawn of *E. coli* and *S. aureus* and creating wells of around 6 µm and using well diffusion method for analyzing its antimicrobial property by comparing its zone of inhibition.

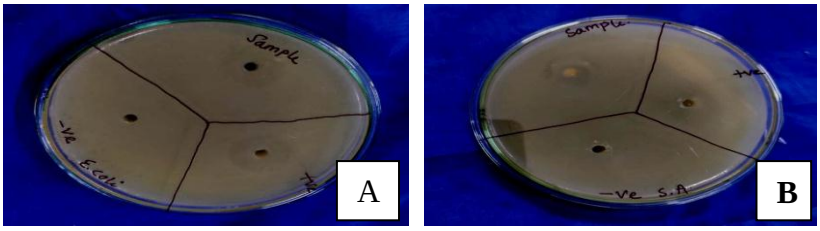

Fig. 9: Halozone seen around positive and sample inoculated in Muller Hinton agar containing A- *E. coli* and B- *S. aureus* for antimicrobial activity.

Table 1: Antimicrobial Test.

Bacteria used	**Zone of inhibition (in mm)**	
	1% streptomycin	**Sample**
Gram+ve bacteria *S.aureus*	10.2	4.3
Gram-ve bacteria *E.coli*	9.0	5.0

Production of different enzymes

For checking for the presence of various enzymes, different tests were done. The assays were performed for enzymes like amylase, protease and cellulase. For the amylase, protease and cellulose activity starch casein agar, skim milk agar and modified Czapekdox-

cellulose agar (4.9gm Czapekdox agar and 1gm of cellulose powder in 100ml distilled water) was used respectively. The fungal culture was streaked and the plates were incubated for 3 days for amylase and protease activity and 5 days for cellulase activity. After incubation the modified Czapekdox-cellulose agar plate and skim milk agar were respectively flooded using Congo red dye and grams iodine. And therefore the activity of enzymes was confirmed by the observation of the halo zones round the colonies in, skim milk agar and can was confirmed by observing the halo zones around the colonies in starch casein agar plates.

DISCUSSION

Since agriculture is import ant for economical progress as well as fulfilling need like hunfer and othr basic comodities the study of Plant disease is an important. Certain Phytopathogenic species of fungi of different genera tend to infect and also cause numerous crops infections, hence cause a great economic loss in agriculture.[20] Fungi are the potential producer of mycotoxin. The mycotoxin is helpful in some cases for contamination but they also possess threat globally to different the food industries and some feed industries also.[21][22] Taxonomy of the genus is complex containing up to 1,000 species with approaches varying between wide and narrow concepts of speciation. Endophytes, some organisms that live in an imperceptible type of relationship with certain plants. The plants in which they reside in a relationship, basically inside the plants, their whole lifetime or a part of it.[23] Endophytes can colonize on all the plants evaluated until now and maybe isolated from almost all of the plant elements like roots, stems leaves, barks, or even from dry seeds.[24] Endophytic fungi are capability producers of diverse elegance of plant-related secondary metabolites with an extensive sort of organic activities which includes antimicrobial agent hypericin.[25] Taxol is anticanceragent[26] and rohitukine also anticancer agent[27] acetylcholinesterase inhibitor huperzine A.[28] From the LPCB mounts we get to know that the grown organism is *Fusarium* because the various fungal structures like mycelia, hyphae sporangia etc resembles with *Fusarium* genera. In our study we also noticed that *Fusarium* greatly affect different types of root vegetables which cause a great economic loss which is very harmful for the economic growth of developing countries like India. We also noticed that *Fusarium* and *aspergillus* genera grow on the host simultaneously. These two genera are the main reason of fruits and vegetables spoilages in different part of the world. *Fusariums* also show certain antibacterial activity which was checked against *E.coli* and *S.aureus*. These two are the main reason of various human diseases, so the genera of *Fusarium* can also be used for the production of

drugs against these two bacteria spp. Our isolate is positive in the production of amylases, protease and cellulases. A scientist, Erhard Friedrich Leuchs who found out that the hydrolysis of starch through saliva, because of the presence of an enzyme in saliva. This is "ptyalin"(old name), and named amylase in the year 1831.[29] [30] It becomes named after the historic Greek name for saliva that is sialon and for ptyalin its miles ptyalon. There are in particular 3 styles of amylases that are α-amylases (EC 3.2.1.1), γ-Amylase (EC three.2.1.3), β-amylase (EC three.2.1.2). Applications of α-amylases are within the starch industry are very extensively spreaded. α amylase enables in the starch hydrolysis and starch liquefaction technique which in turn converts the starch into fructose and glucose syrups[31] (Nielsen and borchert, 2000). The primary purchasers of enzymes are the detergent industries. The usage of enzymes in detergents production enhances the potential of the detergents to take away stains and also make the detergent surroundings friendly without causing pollution. Amylases are the second kind of enzymes that are used in the production of detergent. About ninety% of all liquid detergents comprise amylases as a prime supply.[32] [33] [34] Ethanol is one of the most applied liquid bio-fuels. For the ethanol manufacturing, the most extensively used substrate, starch, due to some unique traits including its low rate and easy availability of raw fabric in maximum regions, also globally.[35] Within the food enterprise the applications of amylases encompass baking, brewing, practise of digestive aids, manufacturing of desserts, fruit juices and starch syrups.[35] Desizing is one of the main approaches within the textile industries. On these desizing method, amylases are the capacity workers. Before fabric manufacturing starch are implemented into yarn as a sizing agent to make a certain, quick and secure weaving process. Starch is a completely appealing sizing agent due to a few unique traits which are cheap, easy availability in most regions and it may be eliminated pretty effortlessly. Starch is later eliminated from the woven cloth in a wet-process inside the textile finishing industry. The process of desizing entails elimination of starch from the cloth. Starch serves because the strengthening agent which prevents breaking of the warp thread in the course of the weaving technique. The α-amylases get rid of selectively the sizing agent and do not attack the fibers of the textile.[36] [37] The α-amylases also are used considerably within the pulp and paper industries. Here amylases are used for the amendment of starch of lined paper for the manufacturing of low-viscosity, high molecular weight starch.[38] Microbial proteases are hydrolytic enzymes. They were studied considerably for the reason that advancement of enzymology research. presently, there is a new interest in the take a look at of proteolytic enzymes, popularity that these enzymes do no longer only play an essential function in mobile metabolic strategies however also due to the fact they've won great interest

in due to the fact they may be industrially vital enzymes.[39] Microbial proteases are labeled into three organizations, primarily based on whether or not or no longer there activation capacity under acidic, impartial, or alkaline environment.[39] The extracellular proteases produced with the resource of microorganisms that are launched out within the booming media have big applications in numerous commercial and commercial fields, but, only a few proteases generating microbes are appeared as business manufacturers of the protease enzyme.[39] The enzyme, proteases generally have packages within the detergent and industrial meals industries. Proteases for industries are prepared in bulk quantities and used as crude samples, whilst proteases with medical packages are synthetically synthetic in small portions and are drastically purified.[40] Proteases have their good-sized programs inside the agency, medicine and they may be drastically applied as an essential organic research device.[41] [42] Cellulases are the magnificence of enzymes which might be composed of independently folding, structurally, and functionally precise units referred to as domains or modules.[43] Cellulases are the enzymes which have been available commercially and drastically for extra than 3 decades, and people enzymes have been used for each instructional and moreover in industrial research.[44] [45] Cellulases also are broadly utilized in pulp and paper corporations. The application of different kinds of cellulases within the pulp and paper company has extended appreciably during the last 10 years.[46] Cellulases are also utilized in material wet processing, those are the most fulfillment enzymes in this kind of industry, the one's enzymes are in particular finishing of cellulose-primarily based textiles, the number one intention is to enhance the advent of the textile.[47] [48] For bio-gas manufacturing, that is a completely latest concern rely now upon an afternoon's, it is possibly the maximum famous software of cellulases enzymes presently being investigated extensively.[49] Microbial cellulases perform an essential role in fermentation techniques at the same time as producing alcoholic drinks like beers and wines.[50] The enzyme cellulases have very enormous and potential programs in food manufacturing industries. The production of fruit and vegetable juices requires advanced strategies for the extraction of juices from their respective fruits and vegetables. Cellulases act as a critical difficulty of macerating enzymes complex. The complicated consists of 3 high-quality enzymes which may be cellulases, xylanases, and pectinases.the maceration enzyme complicated used for extraction and clarification of fruit and vegetable juices, which is beneficial for an increase in the yield of juices.[51] [52] Cellulase enzymes actually have a big variety of applications within the feed industry, additionally, the enzyme named hemicellulase is quite crucial in this example. The ones 2 enzymes have obtained giant attention due to their capability to decorate the price of

feed and ordinary overall performance of animals.[53] distinctive combinations of various styles of enzymes like cellulases, hemicellulases, and pectinases have huge applications in agriculture, it's miles used for enhancing the growth of flora and controlling remarkable kinds of plant ailments.[54] [55] Cellulases also are used to decrease the viscosity of olive paste in olive oil manufacturing. The olive fruit includes polyphenolic substances, the enzyme used to intensify the extraction gadget.[56] The state-of-the-art innovation in the business method is to use cellulases in conjunction with lipase and protease inside the system of detergent production.[45] Within the 21st century, the use of different enzymes in several industries will increase pretty lots. The continuously developing call for enzymes in lots of industries is because of meet call for of suddenly developing population and to save you the extinction of natural resources of numerous compounds.

CONCLUSION

In the study we conducted to isolate the fungus growing on the surface of *the C. longa* and grew it on Czapekdox medium and screened for its morphological and other characteristics like its effect on root vegetables as well as its enzymatic activity. Through this study we confirmed the species as *Fussarium*, which infected the rhizomes of *C. longa* through lacto phenol cotton blue staining. The isolated fungus showed certain antimicrobial activity which was against both *E. coli* and *S. aureus* through well diffusion method. The fungus was able to infect other root vegetables like potato, colocassia, carrot, ginger and onion. On onion the fungus was re inoculated to check its dominance with *Aspergillus* species and found that both grew on onion as black and white colonies were seen simultaneously. Further the isolated fungus also showed various positive results in production of enzymes like amylase, cellulose as well as protease. The fungus isolated from one of the most commonly known spice that is seen to have various antimicrobial and medicinal effects was seen to have various qualities that can make it a possible industrial strain for production of bio based non synthetic enzymes or may be used for its anti microbial properties.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of School of Bioscience and Technology, Vellore Institute of Technology, Vellore- 6032014, Tamil Nadu, for the facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Santos, Ana Carla da Silv et al., Entomopathogenic *Fusarium* species: a review of their potential for the biological control of insects, implications and prospects, Fungal Biology Reviews, https://doi.org/10.1016/j.fbr.2019.12.002.
2. Huiru Zhang, Xinchen Sun and Chunping Xu, 'ANTIMICROBIAL activity of endophytic fungus *Fusarium* sp. Isolated from medicinal honeysuckle plant', Arch. Biol. Sci., Belgrade, 2016; 68(1): 25-30. DOI:10.2298/ABS140401004Z.
3. Azar Hosseinia, Hossein Hosseinzadehb, Antidotal or protective effects of *Curcuma longa* (turmeric) and its active ingredient, curcumin, against natural and chemical toxicities: A review, Biomedicine & Pharmacotherapy, 2018; 99: 411–421.
4. H. Hayakawa, Y. Minanyia, K. Ito, Y. Yamamoto, and T. Fukuda, "Difference of curcumin content in Curcuma longa L., (Zingiberaceae) caused by Hybridization with other Curcuma species," American Journal of Plant Sciences, 2011; 2(2): 111–119.
5. H. P. T. Ammon and M. A. Wahl, "Pharmacology of Curcuma longa," Planta Medica, 1991; 57(1): 1–7.
6. L. Ammayappan and J. Jeyakodi Moses, "Study of antimicrobial activity of aloevera, chitosan, and curcumin on cotton, wool, and rabbit hair," Fibers and Polymers, 2009; 10(2): 161–166.
7. T. Rudrappa and H. P. Bais, "Curcumin, a known phenolic from Curcuma longa, attenuates the virulence of Pseudomonas aeruginosa PAO1 in whole plant and animal pathogenicity models," Journal of Agricultural and Food Chemistry, 2008; 56(6): 1955–1962.
8. N. Niamsa and C. Sittiwet, "Antimicrobial activity of Curcuma longa aqueous extract," Journal of Pharmacology and Toxicology, 2009; 4(4): 173–177.
9. O.-A. Lawhavinit, N. Kongkathip, and B. Kongkathip, "Antimicrobial activity of curcuminoids from Curcuma longa L. on pathogenic bacteria of shrimp and chicken," Kasetsart Journal— Natural Science, 2010; 44(3): 364–371.
10. P. S. Negi, G. K. Jayaprakasha, L. J. M. Rao, and K. K. Sakariah, "Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture," Journal of Agricultural and Food Chemistry, 1999; 47(10): 4297–4300.
11. M. Karaman, F. Fırıncı, Z. Arıkan Ayyıldız, and I. H. Bahar, "Effects of Imipenem, Tobramycin and Curcumin on biofilm formation of Pseudomonas aeruginosa strains," Mikrobiyoloji Bulteni, 2013; 47(1): 192–194.

12. S. A. Marathe, S. Ray, and D. Chakravortty, "Curcumin increases the pathogenicity of Salmonella enterica serovar typhimurium in Murine model," PLoS ONE, 2010; 5: 7. Article ID e11511.
13. L. Tomei, S. Altamura, G. Paonessa, R. De Francesco, and G. Migliaccio, "HCV antiviral resistance: the impact of in vitro studies on the development of antiviral agents targeting the viral NS5B polymerase," Antiviral Chemistry & Chemotherapy, 2005; 16(4): 225–245.
14. I. Dairaku, Y. Han, N. Yanaka, and N. Kato, "Inhibitory effect of curcumin on IMP dehydrogenase, the target for anticancer and antiviral chemotherapy agents," Bioscience, Biotechnology and Biochemistry, 2010; 74(1): 185–187.
15. S. Barthelemy, L. Vergnes, M. Moynier, D. Guyot, S. Labidalle, and E. Bahraoui, "Curcumin and curcumin derivatives inhibit Tat-mediated transactivation of type 1 human immunodeficiency virus long terminal repeat," Research in Virology, 1998; 149(1): 43–52.
16. R. S. Upendra, P. Khandelwal, and A. H. M. Reddy, "Turmeric powder (Curcuma longa Linn.) as an antifungal agent in plant tissue culture studies," International Journal of Engineering Science, 2011; 3(11): 7899–7904.
17. Akter T, Ahmed ATA, Khaleque MA, Begum M. 2013. Effect of drying on quality of tengra (Mystus vittatus) treated with turmeric and salt. Unique Res J Biol Sci., 1: 1e5.
18. Gisele Eleonora Kikot, Roque Alberto Hours and Teresa María Alconada, Extracellular Enzymes of *Fusarium graminearum* Isolates, BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY, July-August 2010; 53(4): 779-783.
19. Eris Septiana, Nampiah Sukarno, Sukarno, Partomuan Simanjuntak Endophytic Fungi Associated With Turmeric (*Curcuma longa* L.) Can Inhibit Histamine-Forming Bacteria in Fish, HAYATI J Biosci (2017), http://dx.doi.org/10.1016/j.hjb.2017.05. 004
20. Aqueveque P, Céspedes CL, Becerra J, Aranda M, Sterner O. Antifungal activities of secondary metabolites isolated from liquid fermentations of *Stereum hirsutum* (Sh134-11) against *Botrytis cinerea* (grey mould agent). Food and Chemical Toxicology, 2017; 109(2): 1048–1054.
21. Kumar KN, Venkataramana M, Allen JA, Chandranayaka S, Murali HS, Batra HV. Role of *Curcuma longa* L. essential oil in controlling the growth and zearalenone production of *Fusarium graminearum*. LWT- Food Science and Technology, 2016; 69: 522–528.
22. Pitt JI, & Hocking AD. Fungi and food spoilage. Dordrecht, Heidelberg, London, New York: Springer. 2009; (3rd ed., p. 519).

23. Wali PP, Wali PR, Saikkonen K, Tuomi J (2013) Is the pathogenic ergot fungus a conditional defensive mutualist for its host grass? Plos One, 8: e69249.
24. Joshee S, Paulus BC, Park D, Johnston PR (2009) Diversity and distribution of fungal foliar endophytes in New Zealand Podocarpaceae. Mycol Res., 113: 1003-1015.
25. Kusari S, /Dmshoi M, Zuhlke S, Spiteller M (2008) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod, 71: 159-162.33- Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of 3Dcific yew. Sci., 260: 214-216. 7.
26. Kumara PM, Zuehlke S, Priti V, Ramesha BT, Shweta S, et al. (2012) *Fusarium* proliferatum, an endophytic fungus from Dysoxylum binectariferum Hook.f, produces rohitukine, a chromane alkaloid possessing anti-cancer activity. Antonie van Leeuwenhoek, 101: 323-329.
27. Kusari S, /Dmshoi M, Zuhlke S, Spiteller M (2008) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod, 71: 159-162. 9. Zhu D, Wang J, Zeng Q, Zhang Z, Yan R (2010) A novel endophytic Huperzine A producing fungus, Shiraia sp. Slf14, isolated from Huperzia serrata. J Appl Microbiol, 109: 1469-1478.
28. Zhu D, Wang J, Zeng Q, Zhang Z, Yan R (2010) A novel endophytic Huperzine A producing fungus, Shiraia sp. Slf14, isolated from Huperzia serrata. J Appl Microbiol, 109: 1469-1478.
29. Leuchs EF (1831). "Wirkung des Speichels auf Stärke" [Effect of saliva on starch]. Poggendorff's Annalen der Physik und Chemie. 22: 623. (Modern citation: Annalen der Physik, 98(8): 623.
30. Pasteur.fr "History of Biology: Cuvier, Schwann and Schleiden", 8 April 2002. Archived from the original on 24 September 2015. Retrieved 17 May 2015.
31. Nielsen J.E., Borchert T.V. Protein engineering of bacterial alpha-amylases. Biochim Biophys Acta., 2000; 1543: 253–274.
32. Gupta R., Gigras P., Mohapatra H., Goswami V.K., Chauhan B. Microbial α-amylases: a biotechnological perspective. Process Biochem, 2003; 38: 1599–1616.
33. Hmidet N., El-Hadj Ali N., Haddar A., Kanoun S., Alya S., Nasri M. Alkaline proteases and thermostable α-amylase co-produced by *Bacillus licheniformis* NH1: Characterization and potential application as detergent additive. Biochemical Engineering Journal, 2009; 47: 71–79.

34. Mitidieri S., Souza Martinelli A.H., Schrank A., Vainstein M.H. Enzymatic detergent formulation containing amylase from *Aspergillus niger*: a comparative study with commercial detergent formulations. Bioresour Technol, 2006; 97: 1217–1224.
35. Couto S.R., Sanromán M.A. Application of solid-state fermentation to food industry- A review. Journal of Food Engineering, 2006; 76: 291–302.
36. Ahlawat S., Dhiman S.S., Battan B., Mandhan R.P., Sharma J. Pectinase production by *Bacillus subtilis* and its potential application in biopreparation of cotton and micropoly fabric. Process Biochemistry, 2009; 44: 521–526.
37. Feitkenhauer H. Anaerobic digestion of desizing wastewater: influence of pretreatment and anionic surfactant on degradation and intermediate accumulation. Enzyme Microb. Technol, 2003; 33: 250–258.
38. van der Maarel M.J., van der Veen B., Uitdehaag J.C., Leemhuis H., Dijkhuizen L. Properties and applications of starch-converting enzymes of the alpha-amylase family. J Biotechnol, 2002; 94: 137–155.
39. Gupta, R., Beg, Q.K. and Lorenz, P. 2002. Appl. Microbiol. Biotechnol., 59(1): 15.
40. Rao, M.B., Tanksale, A.M., Ghatge, M.S. and Deshpande, V.V. 1998. Microbiol. Mol. Biol. Rev., 62(3): 597.
41. *Hooper NM, ed. (2002). Proteases in biology and medicine. London: Portland Press. ISBN 978-1-85578-147-4.*
42. Feijoo-Siota L, Villa TG (28 September 2010). "Native and Biotechnologically Engineered Plant Proteases with Industrial Applications". *Food and Bioprocess Technology*, 4(6): 1066–1088.
43. B. Henrissat, T. T. Teeri, and R. A. J. Warren, "A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants," FEBS Letters, 1998; 425(2): 352– 354.
44. A. Singh, "Engineering enzyme properties," Indian Journal of Microbiology, 1999; 39(2): 65–77.
45. Singh, R. C. Kuhad, and O. P. Ward, "Industrial application of microbial cellulases," in Lignocellulose Biotechnologgy: Future Prospects, R. C. Kuhad and A. Singh, Eds., pp. 345– 358, I.K. International Publishing House, New Delhi, India, 2007.
46. Mai, U. Kues, and H. Militz, "Biotechnology in the wood ¨ industry," Applied Microbiology and Biotechnology, 2004; 63(5): 477–494.
47. Hebeish and N. A. Ibrahim, "The impact of fronteir sciences on textile industry," Colourage, 2007; 54: 41–55.

48. M. Karmakar and R. R. Ray, "Current trends in research and application of microbial cellulases," Research Journal of Microbiology, 2011; 6(1): 41–53.
49. R. Gupta, Y. P. Khasa, and R. C. Kuhad, "Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials," Carbohydrate Polymers, 2011; 84: 1103–1109.
50. R. K. Sukumaran, R. R. Singhania, and A. Pandey, "Microbial cellulases—production, applications and challenges," Journal of Scientific and Industrial Research, 2005; 64(11): 832– 844.
51. R. C. Minussi, G. M. Pastore, and N. Duran, "Potential ´ applications of laccase in the food industry," Trends in Food Science and Technology, 2002; 13: 6-7, 205–216.
52. L. M. J. de Carvalho, I. M. de Castro, and C. A. B. da Silva, "A study of retention of sugars in the process of clarification of pineapple juice (Ananas comosus, L. Merril) by micro- and ultra-filtration," Journal of Food Engineering, 2008; 87(4): 447–454.
53. T. R. Dhiman, M. S. Zaman, R. R. Gimenez, J. L. Walters, and R. Treacher, "Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding," Animal Feed Science and Technology, 2002; 101: 1–4, 115–125.
54. M. K. Bhat, "Cellulases and related enzymes in biotechnology," Biotechnology Advances, 2000; 18(5): 355–383.
55. Riou, Ch.; Freyssinet, G.; Fevre, M. (1992), Purification and characterization of extracellular pectinolytic enzymes produced by *Sclerotinia sclerotiorum*. *Appl. Environ. Microbiol.*, 58(2): 578-583.
56. A. Ranalli, L. Pollastri, S. Contento, L. Lucera, and P. Del Re, "Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing," European Food Research and Technology, 2003; 216(2): 109–115.