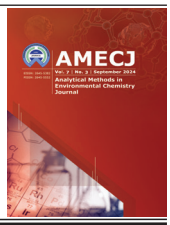
Anal. Methods Environ. Chem. J. 7 (3) (2024) 5-20

AMECJ

Research Article, Issue 3
Analytical Methods in Environmental Chemistry Journal
Journal home page: www.amecj.com/ir



Bioanalytical Method: Determining pesticide residues in tomatoes and mushrooms by a bioactive membrane-based sensor, enzyme inhibition, and UV-Vis Spectrophotometry

Sunita Hooda[a], Ms. Laishram Saya[b], Ms Drashya[c], and Geetu Gambhir[d,*]

[a]*Professor in Chemistry, Acharya Narendra Dev College, University of Delhi, New Delhi, India.*
[b]*Assistant Professor in Chemistry, Sri Venkateswara College, University of Delhi, New Delhi, India*
[c]*Research Scholar, Polymer Chemistry Laboratory, Acharya Narendra Dev College, University of Delhi, New Delhi, India*
[d,*]*Professor in Chemistry, Acharya Narendra Dev College, University of Delhi, New Delhi, India.*

ARTICLE INFO:

Received 1 May 2024
Revised form 15 Jul 2024
Accepted 12 Aug 2024
Available online 30 Sep 2024

Keywords:
Bioanalytical method,
Bioactive membrane based sensor,
Pesticide residues,
Tyrosinase and pyrocatechol,
Enzyme inhibition,
UV-Vis spectrophotometry

ABSTRACT

Pesticides play a vital part in crop production. The present study intends to develop a bioactive membrane-based sensor based on enzyme inhibition for determining pesticide residues. The absorbance (AU) of samples is determined by UV-Vis spectrophotometry. It also aims to study the stability of the enzyme extract at various concentrations. To immobilize the enzyme, different mediums like cellulose, chitosan, guar gum, and cellulose with guar gum are considered for finding an effective membrane. Findings reveal the efficiency of the biosensor in detecting the pesticide residues from tomatoes and mushrooms by finding tomatoes with 0.01M Malathion showed a high absorbance rate of 0.97, 20 times diluted Tyrosinase extract from mushrooms showed maximum absorption of 0.55, and aluminum oxide in tomatoes has explored an absorbance rate of 0.96 at 0.1M concentration. So, the aluminum oxide in tomatoes has explored a high absorbance rate. The LOD, LOQ, RSD, linear range and sensor recovery were obtained for different pesticides. The RSD (%), LOD, and LOQ for these pesticides are obtained at (1.155, 1.81, 2.09 mmol L^{-1}), (3.81, 5.973, 6.897 mmol L^{-1}), and (11.55, 18.8, and 20.9 mmol L^{-1}), respectively. The linear ranges are 2, 6, and 20 mmol L^{-1}. Then, the outcomes of Tyrosinase activity inhibition were obtained through absorbance of different pesticides.

1. Introduction

Pesticide is the common term for the substances utilized for poisoning pests like insects, rodents, weeds, etc. Pesticides that are harmful to human beings are rodenticides and insecticides. Farmers generally use synthetic pesticides due to their simple application, widespread availability, efficiency, and economic returns with more environmental costs. Recently, the residues of toxic pesticides in food samples and groundwater have enhanced public concerns, particularly in association with children's health [1]. Due to the increased utilization of pesticides over the last few years, the possibility of disclosing these toxic chemicals has also found considerable improvement. Currently, the use of pesticides has found significant enhancements in satisfying the demands of people due to rapid population development. This is because more

*Corresponding Author: Geetu Gambhir
Email: geetugambhir6@gmail.com
https://doi.org/10.24200/amecj.v7.i03.255

food has to be produced due to an increase in the number of pesticides used in agriculture in the community It resulted in high farm productivity for which the farmers employed pesticides. Various reports stated the prime pesticide levels in the food could lead to the improvement of diseases such as kidney, lung, and cancer ailments. Based on the reports of the WHO (World Health Organization), every year, nearly 30,00,000 cases of people are getting poisoned by pesticides with 2,20,000 deaths, especially in developing countries [2]. Moreover, 2.2 million individuals, mainly from evolving countries have been exposed to a high risk of pesticides [3, 4]. Traditional research tried to study the detection of pesticide residues. Accordingly, a perspective on using pesticides in the agricultural area has been afforded to find the pesticide's impact on the environment and food production. Hence, it has been vital to emphasize the significance of finding the residues of pesticides in food aiming to confirm food safety as these compounds could indicate the risks associated with the environment and human health [5, 6]. It has also been exposed that the mass spectrometry and chromatographic methods support finding the pesticide residues for assessing the food quality which reached customers along with MRLs (Maximum Residue Limits) laid by each country's legislation, these instrumentations turn to manage the population exposure to pesticides [7]. Though MRL has been utilized as the food quality parameter, global variations in the pesticide legislation do not guarantee the safety of consumers. For the diet of human beings, vegetables, and fruits have been an essential part as they possess essential nutrients needed for a normal human to react to several reactions within their body. Persisting pesticide usage has resulted in several issues including the health of humans. To maintain losses and manage the standardization of these vegetables and fruits, harvest pesticides have been used. The main intention of the conventional research has been to find the quality of pesticides existing in these foods by computing the normalized variation of vegetation index through the sensor. The process has been undertaken in two fragments. The initial part identifies the vegetables and fruits through the usage of a Convolutional Neural Network (CNN) by training it with the image feature sets like shape, texture, and color. In the subsequent module, the pesticides in these vegetables and fruits have been detected through three manners for computing its Normalized Difference Vegetation Index (NDVI) through the usage of a gas sensor and IR sensor. Then, the comparison has been undertaken. Arduino program afforded the output. Information that has been detected will be shown on the screen. Following this, the graph has been plotted. It has been concluded that the gas sensor provides high accuracy for pesticide detection [8]. Analysis has been undertaken to review rapid pesticide detection approaches involving enzyme inhibition, fluorescence sensor, biosensor detection, spectrophotometric and chromatographic analysis, which has examined the progressive status. It has been summarized that the rapid detection method has become faster and highly extensive [9, 10]. A Fluorometric-atrazine assay relying on N-GQDs and Tyrosinase inhibition has been developed. This Tyrosinase includes a copper-comprising enzyme of the animal and plant tissues that catalyzes melanin production and supplementary pigments from the Tyrosine through oxidation like the blackening of sliced or peeled potatoes that have been exposed to air. It has been found within the Melanosomes. In the molecular-biology, Tyrosinase indicates an oxidase that indicates the enzyme limited by rate to manage melanin production [11]. Tyrosinase carries phenol oxidation, such as pyrocatechol, using di-oxygen and Tyrosine. In Tyrosinase existence, benzoquinone has been formed from the Cathecol, and the structure of Tyrosinase is shown in Figure 1.

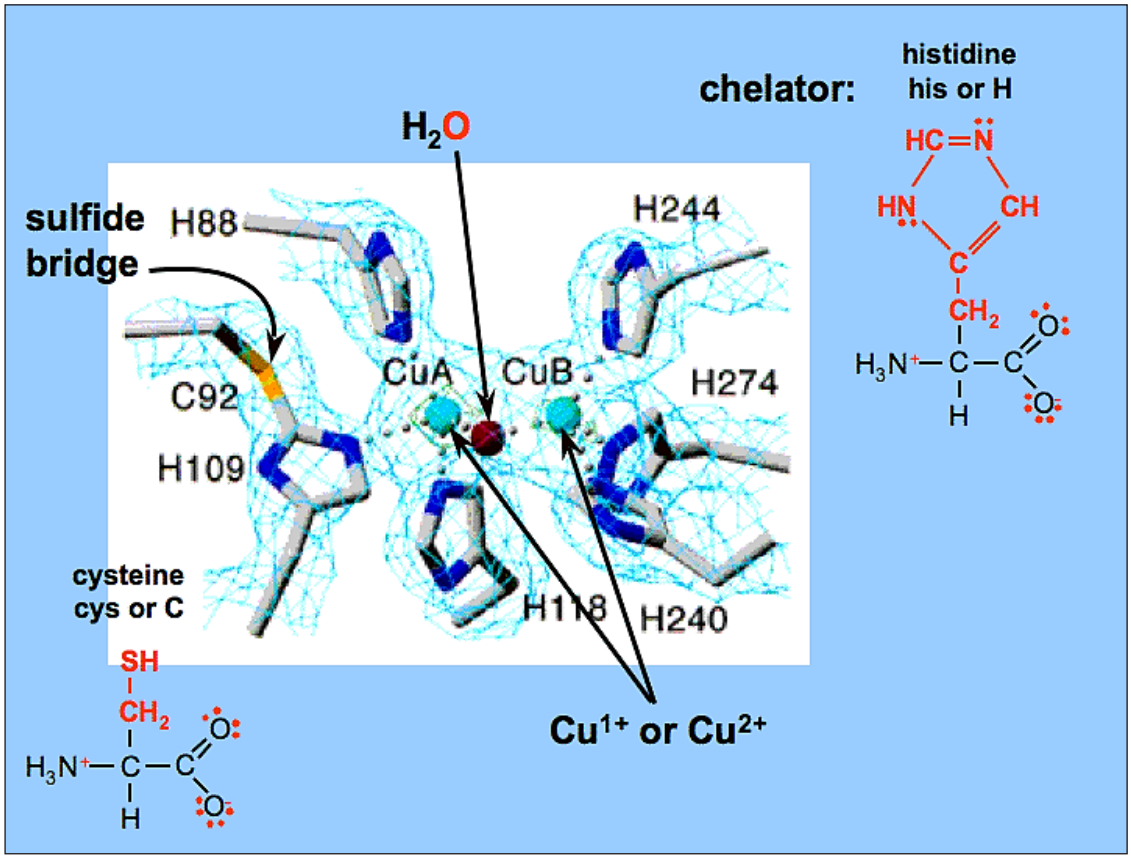


Fig.1. Structure of Tyrosinase

NGQDs have been synthesized through hydrothermal reaction by applying ammonia and citric acid. The tyrosinase-driven dopamine oxidation reaction produced dopaquinone, which could effectively reduce N-GQDs fluorescence by the dynamic quenching method. Tyrosinase inhibitor named atrazine could alleviate enzymatic reaction by declining the Dopaquinone generation resulting in fluorescent recovery that depends on the atrazine concentration. The recent identification of the metabolite or parent compounds has impacted the pesticide analysis rate, thus instigating the request for methods to compute minimum concentration levels [12].

In addition, current knowledge regarding the detection and isolation of pesticides in fatty commodities has been analyzed. Several isolation methods have been discussed such as solvent partitioning, dispersive solid-phase, and solid-phase, matrix solid-phase dispersion, gel-permeation chromatography, accelerated-solvent extraction, micro-extraction methods, Soxhlet extraction, and QuEChERS-based methods. The chromatographic techniques pre-dominate the pesticide residue analysis in the fatty matrices. Due to this, the analysis concentrates on these methods, especially those integrated with mass spectrometry. Still, analytical chemists face issues in determining pesticide residues in the fatty matrices to develop quick and simple ways that consume minimum organic solvents. Until now, various methods have been introduced to extract and detect pesticides from conventional to progressive detection methods [13-15]. A comprehensive review of accessible traditional methodologies (liquid chromatography integrated with several detectors and gas chromatography) to advanced pre-treatment (magnetic nano-particle coated with polystyrene) and detection (nano-technology and sensor development) methods utilized in pesticide residue analysis in several vegetables and fruits have been analyzed.

Though the detection and extraction systems options remain similar, these methods have been expensive and time-consuming [16]. In recent years, advanced methodologies like biosensors [17], nanotechnology, and molecular-imprinted polymers have been used as an alternative to detect pesticides. Progressive enhancement of biosensors has been explored to detect dichlorvos pesticides, from the usage of traditional immobilizing assistances to highly promoted composite or hybrid nanomaterials. Further, biosensor development has been summarized through enzyme inhibition methodologies wherein enzymes like Tyrosinase, AChE (Acetylcholinesterase), etc have been immobilized on transducer by conventional immobilization methodologies or applying advanced nanomaterial to integrate in a better manner [18]. A six-stage method has been explored for designing the enzyme sensors aimed at evaluating the complex matrix quality. This has to be followed to accomplish maximum probable biosensor sensitivity to probable toxic substances for reducing the impact of uncontaminated complex mixture elements on the biosensor activity. Testing has been performed with the endorsed approach to outline a bio-luminescent biosensor to integrate rapid evaluation of vegetables and fruit safety. Techniques and methods have been described to attain the desired outcome in the individual stage. It has been found that the six-stage technique to design the bio-luminescent enzyme biosensors could be utilized for designing the enzyme-based sensors relying on several other enzymes [19]. Traditional studies were undertaken on biosensors to inhibit enzymes to find the carbamate pesticides and organophosphorus compounds, which have been reviewed [10, 20, 21]. Biosensor sensitivity has been enhanced by including effective immobilization methodologies like thin-polymer films and self-assembled monolayers. Additionally, a computational model framed from perceived data relies on varied inhibition approaches with diverse inhibitors that assist to classify and find pesticides in real-time samples concurrently. Likewise, critical analysis has been emphasized between 2015 and 2019 along with to date overview of analysis methodologies and extraction techniques in detecting the residues of pesticides in several food samples [22]. Theoretically, pesticide residues existing in food might metabolize and generate new chemical substances while processing food. Thus, research is essential for biosensor development that could be utilized for detecting the pesticide's existence during the pre-processing stage [23]. Consequently, pesticide detection through biosensor technology has been a promising area, and efficient products are expected to be developed for huge employment in the future [24, 25]. It has also been vital to estimate the existence of these toxic chemicals in daily food constituents beyond the tolerable limit. Also, the organic compounds such as VOCs and pesticides can be determined by different techniques such as gas chromatography, UV-Vis and HPLC in different matrixes [26-31]. To attain efficient knowledge, the present study aims to generate enzyme-based bioactive sensors for sensing the existence of pesticides in fruits and vegetables consumed regularly. To accomplish this, the study considers extracting Tyrosinase (enzyme) from food samples. In this study, tomatoes and mushrooms are considered as samples and the enzyme inhibition rate with various metal inhibitors and pesticides is researched to find the effectiveness of the system in the detection of pesticide residues.

2. Experimental

The research aims to develop bioactive membrane-based sensors to determine pesticide residues. Though conventional works attempted to detect pesticide residues, they have been ineffective and time-consuming. Thus, the present work performs experiments based on the below steps to prepare the membrane. Absorbance (AU) and transmittance (%) are measurements used in spectrophotometry (Cary 4000, Agilent, USA). Spectrophotometry UV-Vis measures how much radiant energy a sample absorbs at varying wavelengths of light between 175-900 nm. The UV-Vis technique determined the concentration of pesticide residues in solutions after calibration by standards.

2.1. Experimental procedure to prepare membrane

In this section, tyrosinase from two detected sources tomato and mushroom were extracted. The stability of enzyme extraction at varied pH and temperatures was also studied. In addition, immobilizing the enzyme extracts on different mediums such as chitosan, guar gum, and cellulose was done and the integration of the two in diverse ratios as well as its standardization to use further. The enzyme activity with diverse indicators utilizing the colorimeter studied and enzyme inhibition through different metal ions and pesticides with the utility of standardized indicators was obtained. Casting an immobilized enzyme membrane on ideal solid support was evaluated.

2.1.1.Extraction of Tyrosinase

2.1.1.1.Extraction of Tyrosinase from tomato

First, 300 g of tomatoes are added to a blender with 300 mL of sodium fluoride (NaF). Then, a tomato is peeled and cut into 1-inch squares. After this, it is homogenized for one minute at high speed. The mixture (homogenate) is poured through various cheesecloth layers into the beaker. The volume of the obtained mixture is measured. Following this, an equal amount of aluminum sulphate is added. This led to the appearance of a fluffy white precipitate as several early soluble proteins of tomato became insoluble. A tyrosinase enzyme is one of these proteins, thus, it is found later in the precipitate [32]. The above mixture is partitioned into chilled centrifuge tubes. This is then centrifuged at 3000rpm at 4°C. Subsequently, centrifuge tubes are carefully collected and poured off. Then, the fluid is discarded and the pellets are saved. All pellets are integrated into a 100ml beaker. A citrate buffer of 60 mL is integrated into the pellets. Contents are stirred well. Following this, a glass rod is utilized for breaking the pellet to preserve its coolness. This solution is again partitioned into the centrifuge tubes. This is again centrifuged at 3000 rpm for five minutes at 4°C. The supernatant is gathered and then saved. It includes the extracts of the enzyme. This is then positioned in volumetric flasks and the enzyme extracts are labelled and placed in the ice bucket.

2.1.1.2.Extraction of Tyrosinase from Mushrooms

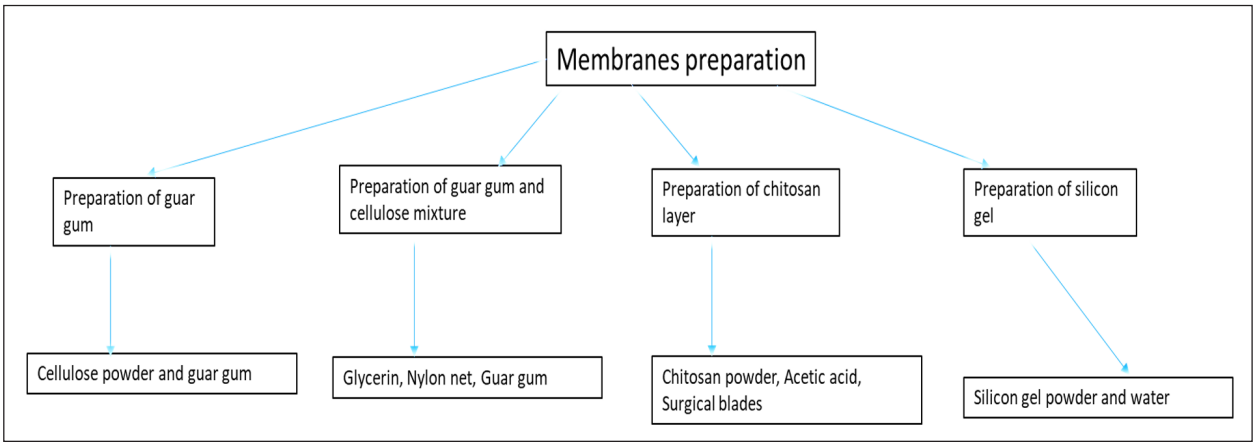
Mushrooms are dissected into small parts. It is then weighed for 30 g. Each mushroom cube is wrapped into a separate [33] Aluminium foil. After some time all the foils are removed and the mushrooms are put into a blending flask. 50 ml of the Tris solution containing NaF is added and blended for 1 minute and 3 times. The blended mixture is filtered through a fritted funnel and the filtrate is fed into a conical flask. The mouth of the flask is covered with Aluminium foil and immediately put into an ice bucket full of ice.

2.1.2.Study of enzyme extraction stability at varied pH

To study the stability of enzyme extraction at different pH and temperatures, a Tris buffer is made with NaF solution based on the below procedure[34]. We used this powder (0.605 g), HCl (1 N), NaF solution (0.1 M), and distilled water. To prepare 1 N HCl, we added 11 ml distilled to 1 mL HCl. The preparation of 0.1 M NaF solution was done by adding 0.295 g of NaF to 50 ml of distilled water. So, the tris solution prepared by 2 gm of tris dissolved in 50 ml distilled water and the final tris solution containing NaF prepared by 12 ml HCl solution is added to the prepared Tris solution. The above mixture is Tris buffer. Then, 25 ml of Tris buffer and 25 ml of 0.1 M NaF solution are taken in a conical flask and mixed correctly. This has given 50 ml of the above solution.

2.1.3.Preparation of membranes

Due to Schema 1, membranes like guar gum, cellulose, chitosan, and a silica gel layer are also prepared to stabilize the enzyme activity. This preparation is achieved using the procedure below.



Schema 1. Preparation of membranes like guar gum, cellulose, chitosan, and silica gel layer

2.1.3.1. Preparation of guar gum

The guar gum is prepared with 2 g of guar gum powder which is weighed and put in a 400 ml beaker and 4 ml of Isopropanol is added to it and stirred properly. Then, 200 ml of distilled water is added and stirred vigorously until dispersed uniformly. The mixture is divided into two parts into two separate beakers. One beaker is placed in a water bath for 10 minutes. After ten minutes, it is labelled as a heated one and another mixture remains as such.

2.1.3.2. Preparation of guar gum and cellulose mixture

In the mixtures, the cellulose powder is added and it is mixed well so that the cellulose gets mixed completely with guar gum. The consistency is checked according to the below procedure.

2.1.3.3.Preparation of guar gum – cellulose membrane

The guar gum-cellulose membrane is prepared by a ratio of 3:2. A few glass slides are taken, and the nylon net is cut into small pieces of slide dimensions. Then, some glycerin is spread over the glass slides completely. The nylon net is kept on each slide and the guar gum and cellulose mixture are spread over the slides containing the nylon net. Then, it is left for 24 hours to dry, and the formed membrane is finally removed.

2.1.3.4. Preparation of chitosan layer

The chitosan layer is prepared with 2 g of chitosan powder is weighed and put into the beaker. Then, 50 ml of the distilled water is added and the chitosan powder is dissolved properly in it and 1 ml of Acetic acid is added to it which makes the mixture viscous. At that moment, it is stirred for half an hour until all the bubbles are removed from it. Then, the mixture is spread in a petri dish and left undisturbed for 24 hours for drying. Finally, it is removed through surgical blades from a Petri dish.

2.1.3.5.Preparation of silica gel layer

The silica gel layer is prepared using some amount of silica gel powder and added to distilled water. It has been mixed until the required consistency is obtained. If required, silica gel or water is added to it. Then, the above mixture has been spread over the glass slides. It is kept undisturbed until it dries completely. Finally, the silica gel layer is ready for further tests [35]

2.1.4. Immobilization of the enzyme extracts

Immobilization indicates the enzyme attachment [36] onto an inert and insoluble material that could afford high resistance to alterations in different conditions like temperature or pH. This permits enzymes to hold it throughout the reaction, this is followed by easy separation from corresponding products that might be utilized again. It is an effective method and is widely employed in industries for catalyzed reactions of the enzyme. The immobilized enzymes are highly significant for commercial usage due to their benefits for

processing reactions. The main advantages of these enzymes are economy, convenience and stability. As an economic advantage, the immobilized enzyme could be easily partitioned from the reaction making it easy to recycle the bio-catalysts. For convenience, the small quantities of protein get dissolved in reactions so that the workup could be easier. After completion, the reaction mixture usually comprises only the reaction and solvent products. As a stability advantage, the immobilized enzymes possess high operational and thermal stability in comparison to soluble immobilization which indicates the enzyme attachment to the inert and insoluble material. It could afford enhanced resistance to alterations in conditions like temperature or pH. This permits enzymes to be detained in a position throughout the entire reaction. Then, these are separated easily from products and enzyme forms. Also, three effective approaches exist for enzyme immobilization adsorption, entrapment and cross-linkage. Due to adsorption, attachment of enzyme to the exterior of inert material occurred. Generally, this methodology is slower than the entrapment and entrapment process. As absorption is not a chemical reaction, the active site corresponding to the immobilized enzyme might be blocked through a bead or matrix greatly minimizing the enzyme activity. The structure of Tyrosinase exploring its active site is shown in Figure 2.

Two copper atoms within the active site of the Tyrosinase enzymes communicate with the di-oxygen to form highly reactive chemical intermediation that oxidizes the substrate. Tyrosinase activity is identical to the catechol oxidase that is associated with the copper oxidase class which is collectively called polyphenol oxidase. The other effective approach for enzyme immobilization is entrapment. In the entrapment, the enzyme is confined to the microspheres or insoluble beads, such as a calcium alginate reaction. Nevertheless, this insoluble material hinders the substrate arrival and product exit. Also, cross-linkage is another effective approach for enzyme immobilization.In the cross-linkage, covalent bonding of enzyme to the matrix is performed by a chemical reaction. It is more

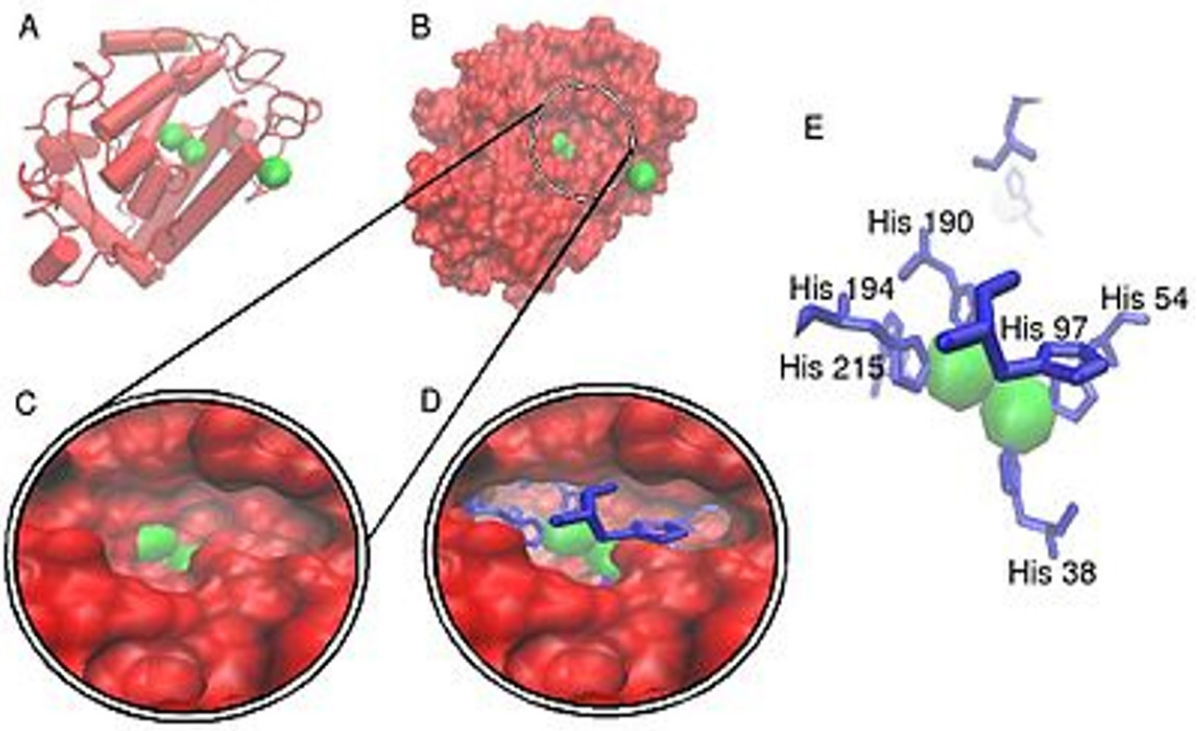


Fig. 2. Active sites of tyrosinase structure

efficient than entrapment and absorption. Chemical reaction confirms that the binding site does not cover the active site of the enzyme, enzyme activity is only impacted by immobility. Nonetheless, covalent bond inflexibility impedes self-healing features explored through the chemo-absorbed and self-assembled monolayers.

2.1.5.Immobilization of the Tyrosinase extract on the different layers

The layers prepared (chitosan, guar gum, cellulose, and integration of two in diverse ratios) are then embedded with Tyrosinase by pouring the enzyme extract of the most active concentration over them [37]. It has been left undisturbed at a low temperature for 10 -12 hrs. The enzyme gets adsorbed over the layer to retain its properties. So, chitosan, guar gum, and cellulose are explained below.

Chitosan is a functional linear polysaccharide that can be produced from the N-Deacetylation of chitin. It is a biopolymer comprised of N-acetyl glucosamine and glucosamine units comprised of glycosidic linkages. Due to the amino groups on the polysaccharide chain, the chitosan could be positively charged and solubilized when the pH of the solution is below 6, thus turning into a polycationic polymer. The chitosan gets despoiled into the non-toxic product in the Vivo. Hence, it is widely utilized for several biomedical applications. The feasibility of using an acid solution such as acetic acid for chitosan dissolution and the subsequent homogeneous, porous membrane is produced.

Chitosan membrane works as a very good adsorptive platform for the enzyme Tyrosinase and is cross-linked with it. Tyrosinase is stable in the membrane for more than 15 days at 4°C (in the fridge). On reacting with Pyrocatechol, the immobilized enzyme in the chitosan membrane turned dark due to Ariel oxidation. The molecular structure of chitosan is shown in Figure 3 and the formation of chitosan from chitin is explored in Figure 4.

Cellulose is a polysaccharide comprised of long chains of the linked with the units of D-glucose. This is utilized in huge application ranges that need a structured backbone. It is employed in preparing the chromatographic structural media in the gels to process like ionic separations and electrophoresis as well as a substrate for identifying and studying the cellulosic systems. The molecular structure of cellulose is shown in Figure 5.

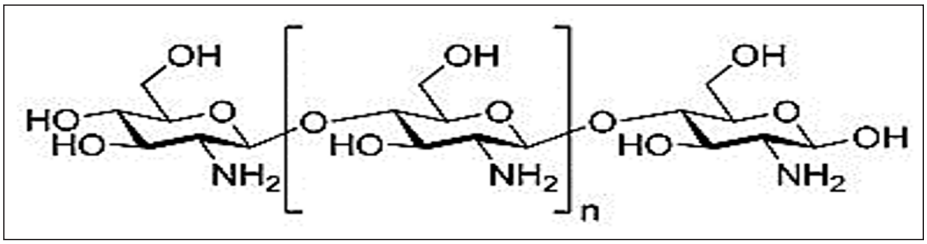


Fig. 3. Molecular structure of chitosan

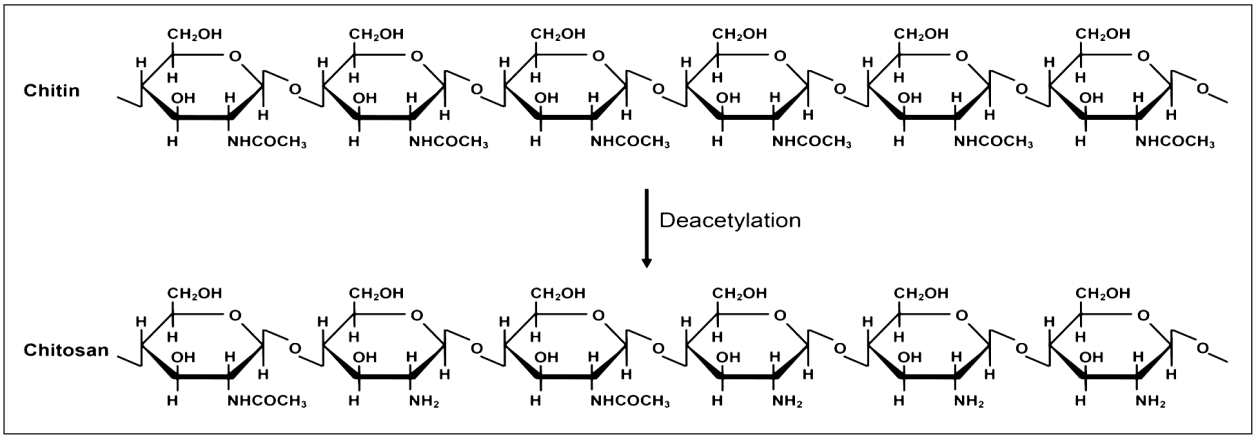


Fig. 4. Formation of chitosan from chitin

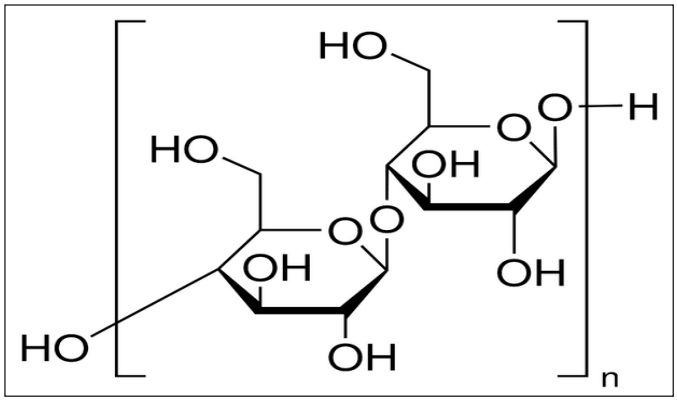


Fig. 5. Molecular structure of cellulose

The paper-based product features usually comprise the cellulose fibres of 90 to 99% that are the fundamental structural component with significant components impacting the properties of end usage. A Paper network is comprised of the laid fibrous and non-fibrous materials that comprise complicated cavity pore channel sets with several capillary dimensions. Thus, it is possibly permeable to the liquids. Nevertheless, the paper structure could be altered during the liquid contact as it interrupts the hydrogen bonds, and fibre relaxation and generates dimensional alterations in the capillaries and pores. So, cellulose can be used to make a membrane on which enzymes can get adsorbed and can be checked for its activity. The drawback of cellulose powder is, that it doesn't bind easily to form a membrane, thus, guar gum is prepared to add viscosity and bind cellulose powder. It makes the formation of the membrane possible and provides a white background to it. To make the film stiff, guar gum and cellulose solution are transferred to nylon mesh.

2.1.6.Inhibition of the enzyme by the pesticide

The presence or absence of the pesticide could be tested only if the immobilized enzyme on the membrane gets inhibited by the known pesticide [38]. Solutions for different pesticides like Malathion, Carbofuran, and aluminium phosphide in different concentrations are prepared and the membranes are dipped in these solutions for various recorded times. These are then taken out at the noted time intervals and tested for enzyme activity using the indicator (developer). The inhibition of the enzyme is more with lesser qualitative color intensity produced with the indicator. In this work, Pyrocatechol is utilized as an indicator for Tyrosinase activity as it oxidizes the Tyrosinase thereby showing the color change.

2.1.7.The casting of the membrane

The immobilized Tyrosinase on different layers is then cast into a stable membrane by initially pouring the solution of these inert layers on the fine nylon mesh. It is made to dry completely and the extract of Tyrosinase is adsorbed over the membrane for 10-12 hrs. Finally, the casted membrane [39] is ready to use.

3. Results and Discussion

Observations on absorption rate at various concentrations of a complex formed by Tyrosinase, enzyme activity at different concentrations, the activity of Tyrosinase membrane with various metal inhibitors, absorption of tomato extracts with pesticides with Tyrosinase membrane using Pyrocatechol as an indicator, and inhibition of Tyrosinase activity by different pesticides absorbance are discussed in this section. Pyrocatechol has been used as an indicator in this context. Initially, the results were obtained for the absorbance of Pyrocatechol at various concentrations of 1M Pyrocatechol solution, 0.1M Pyrocatechol solution, 0.2M Pyrocatechol solution, and 0.3M Pyrocatechol solution are shown in Table 1 and Figure 6.

Table 1. Pyrocatechol and its absorbance rate at various concentrations

Solutions	Absorbance
1M Pyrocatechol solution	a.u 0.1
0.1M Pyrocatechol solution	a.u 0.17
0.2M Pyrocatechol solution	a.u 0.06
0.3M Pyrocatechol solution	a.u 0.02

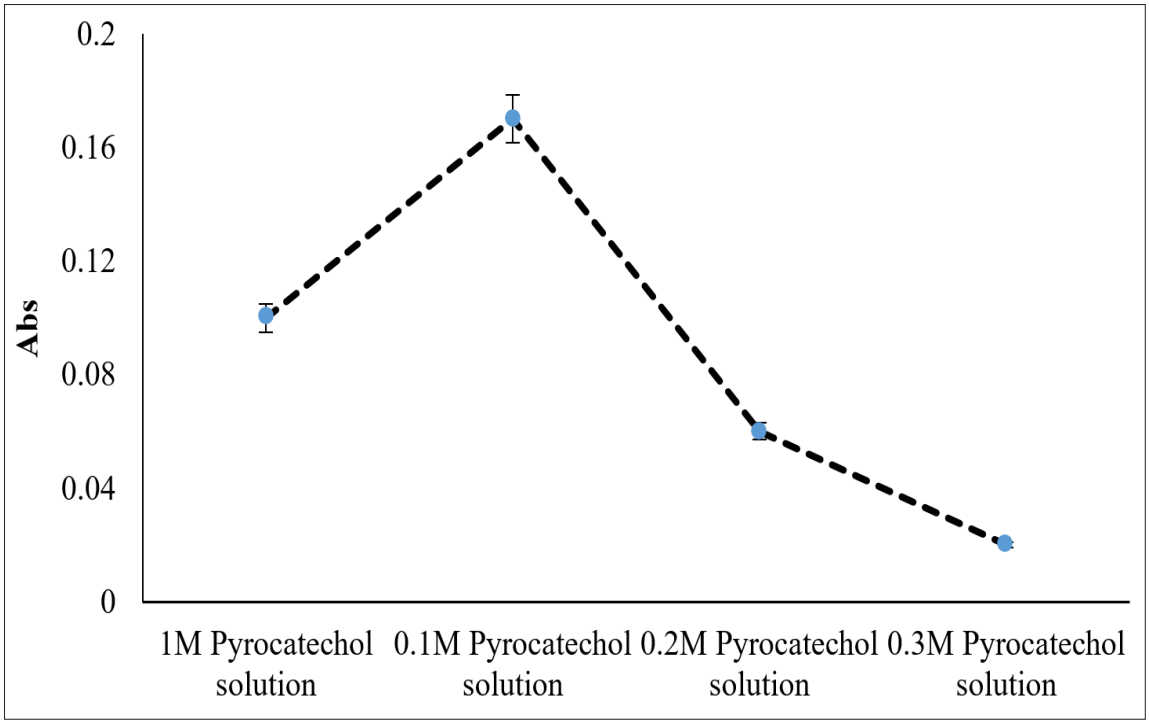


Fig. 6. The absorbance of Pyrocatechol at different concentrations

From Figure 6, the maximum absorption was observed for a 0.1M solution of Pyrocatechol with the enzyme extract. Henceforth, this indicator concentration would be used to study the enzyme activity at several concentrations. The obtained outcomes are shown in Table 2 and Figure 7. The absorbance of the sensor must be checked with a high instrument for the first time. The SD value is 1.155 and the LOD (limit of detection) and LOQ (limit of quantity) values are 3.8115 and 11.55 mmol L^{-1}.

From Figure 7, the absorbance curve for commercial and extracted Tyrosinase gave a better concentration estimate when extracted from the mushroom. That is, 20 times diluted Tyrosinase extract from mushrooms showed a maximum absorption of 0.55 and hence it could be considered for further use. Further, the activity of the Tyrosinase membrane with different metal inhibitors has been studied using the Pyrocatechol indicator. Obtained results are shown in Table 3 and Figure 8. The SD value is 1.81 and LOD (limit of detection) and LOQ (limit of quantity) values are 5.973 and 18.1 mmol L^{-1}

Lead acetate, lead nitrate, and aluminum sulphate are metal inhibitors. It has been concluded that a major change in the colour of the Tyrosinase membrane indicated the inhibition of the Tyrosinase by the salts of Aluminium and Lead. Tyrosinase produces a brown colour when reacted with Pyrocatechol. However, the colour formation (activity of the enzyme) was inhibited by different

Table 2. Study of enzyme activity at different concentrations using 0.1 M Pyrocatechol in mushroom

Tyrosinase concentrations	Absorbance
Stock Solution (0.1M)	0.66 a.u
0.001M Tyrosinase(commercial)	0.6 a.u
0.002M Tyrosinase	0.04 a.u
0.003M Tyrosinase	0.01 a.u
Mushroom Tyrosinase (40 times diluted)	0.34 a.u
Mushroom (30 times diluted)	0.43 a.u
Mushroom (25 times diluted)	0.49 a.u
Mushroom (20 times diluted)	0.55 a.u

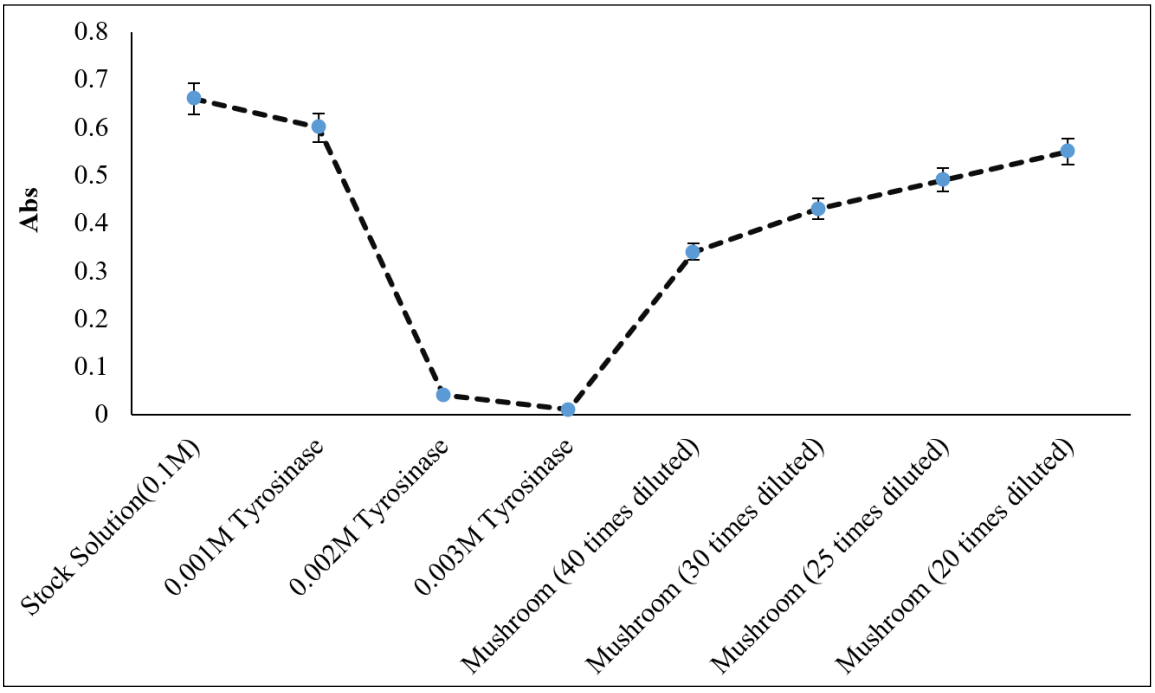


Fig. 7. Absorbance rate at different concentrations of the Tyrosinase

Table 3. The activity of the Tyrosinase membrane with various metal inhibitors using the Pyrocatechol (indicator)

Pyrocatechol + TM	Dark Purple
Pyrocatechol+ TM+ lead acetate	Green
Pyrocatechol +TM +lead nitrate	Light green
Pyrocatechol + TM + aluminum sulphate	Brown
TM: Tyrosinase Membrane*	

pesticides. Malathion inhibited the enzyme's activity when it was added to the film embedded with Tyrosinase which produced no colour with the addition of Pyrocatechol. Moreover, outcomes were attained through the addition of Malathion on Tyrosinase. Carbofuran also inhibited the activity of the enzyme as it also did not produce any colour change with Pyrocatechol. Aluminum oxide also inhibited the activity of the enzyme as it also did not produce any colour with Pyrocatechol. Further, a chitosan membrane was prepared which proved to be a perfect immobilization material for Tyrosinase, but the problem was the lack of a light background. And the membrane once air-dried became very stiff. Once the enzyme gets embedded in the addition of Pyrocatechol it turns dark in colour (blackish). While inhibition of enzymes with pesticides showed no colour change. Then, silica gel mesh was prepared on glass slides, on which Tyrosinase was embedded, it provided a very fine background and colour change was observed with the addition of Pyrocatechol in the immobilized enzyme as yellow which showed brown colour when inhibitor (pesticides) were present as shown in Figure 8. However, the enzyme was not stable for more than three days.

Then, attempts were made to work with cellulose, as its light colour gives a good background and enzyme easily and on the addition of indicator it showed a dark purple colour. While, in the presence of pesticides, it gives a brown colour. The drawback of cellulose was, the inability to bind. Thus, it was mixed with guar gum solution to make a good quality membrane. So, the guar gum–cellulose membrane was found to be effective. Then, the results of the absorption rate of tomato extracts with different pesticides are shown in Table 4 and Figure 8.

Table 4. Study of absorption of tomato extracts with different pesticides with Tyrosinase Membrane using Pyrocatechol as an indicator

Solutions	Absorbance
Fresh Tomato	a.u 0.35
Tomato in Carbofuran (0.1M)	a.u 0.46
Tomato in Malathion (0.01M)	a.u 0.32
Tomato in Al_2O_3 (0.1M)	a.u 0.96

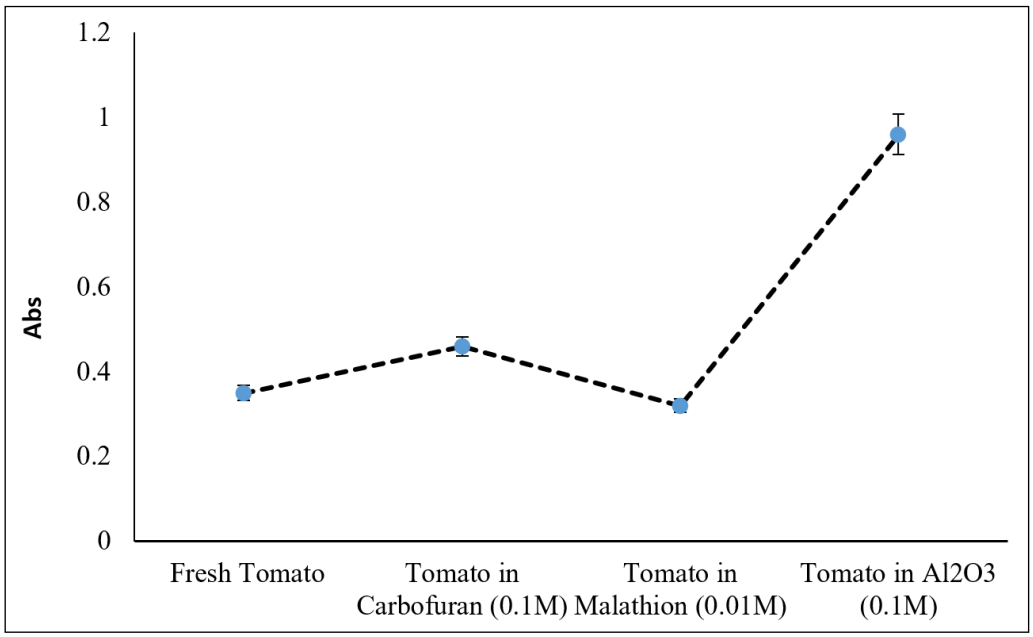


Fig. 8. The absorption rate of tomato extracts with different pesticides

The absorbance of tomato extract with different pesticides was determined using a biosensor. According to Figure 8, it is found that tomatoes in aluminum oxide have explored a high absorbance rate of 0.96 at 0.1M concentration. Then, the outcomes obtained for inhibition of Tyrosinase activity through absorbance of different pesticides are shown in Table 5 and Figure 9. The SD value is 2.09 and LOD (limit of detection) and LOQ (limit of quantity) values are 6.897 and 20.9 mmol L^{-1}. The absorbance of the sample using UV-Vis is shown in Table 5.

The inhibition of Tyrosinase activity in different pesticides is identified using biosensors. 0.01546 mmol L^{-1} is the value for absorbance 0.3, 0.7 absorbance attained 0.0608 mmol L^{-1}, absorbance of 0.97 value has 0.05000 mmol L^{-1}, 0.69 absorbance value obtained 0.03557 mmol/L and 0.66 absorbance attained 0.03402 mmol L^{-1} for cell length 1 and molar absorptivity 19400 for all the absorbance.

From Figure 9, it is found that the extract of tomato with 0.01M Malathion showed a high absorbance rate of 0.97, while, Tyrosinase + Tomato extract with pesticide (Malathion)+ Pyrocatechol showed a minimum absorbance rate of 0.66. Thus, it could be concluded that 20 times diluted Tyrosinase extract from mushrooms showed maximum absorption of 0.55, and tomato in Aluminium oxide has explored a high absorbance rate of 0.96 at 0.1M concentration. The 0.01 molar value in mg L^{-1} is 980 for a density of 0.001 and molecular weight of 98 mg.

4. Conclusion

The research aimed to develop bioactive membrane-based sensors through enzyme inhibition for to determine pesticide residues, especially in tomatoes and mushrooms. The stability of the

Table 5. Inhibition of Tyrosinase activity through the absorbance of different pesticides

Solutions	Absorbance
Tyrosinase + Pyrocatechol	a.u 0.3
Extract Tomato without Pesticide	a.u 0.7
Extract Tomato with 0.01M Malathion	a.u 0.97
Tyrosinase + Tomato extract without pesticide + Pyrocatechol	a.u 0.69
Tyrosinase + Tomato extract with Malathion+ Pyrocatechol	a.u 0.66

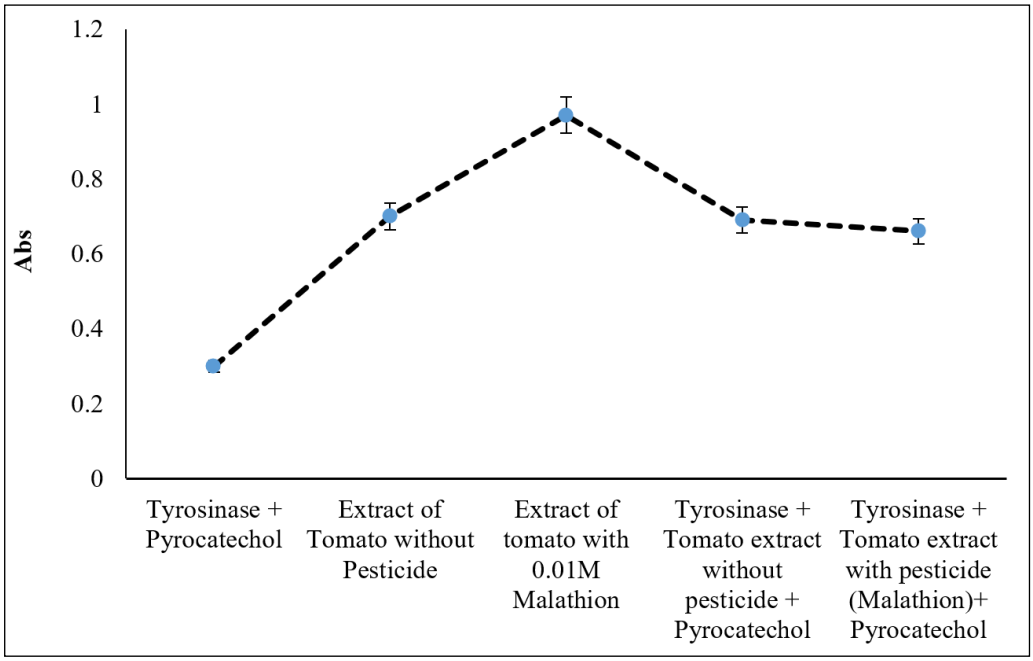


Fig. 9. Inhibition of Tyrosinase activity by different pesticides

enzyme extract at different concentrations was studied. Immobilization of the enzyme extract on different mediums like cellulose, chitosan, guar gum, and a combination of guar gum and cellulose was tested. Due to the effective binding and stable nature of guar gum and cellulose, it was integrated and considered a membrane. Pyrocatechol was used as an indicator for Tyrosinase activity as it oxidizes Tyrosinase by showing the colour change. The maximum absorption was observed for a 0.1M solution of Pyrocatechol with the enzyme extract. Thus, this concentration of the indicator was used to study the enzyme activity at several concentrations. Finally, the study was undertaken for the inhibition of an enzyme by different pesticides and metal inhibitors with the use of 0.1M Pyrocatechol. It was found that 20 times diluted Tyrosinase extract from mushrooms showed maximum absorption of 0.55. Meanwhile, tomatoes with aluminum oxide had a high absorbance rate of 0.96 at 0.1M concentration. This maximum rate of absorption indicates the efficiency in detecting the pesticide residues. The LOD, LOQ, RSD%, and linear range (LR) and recovery of the sensor were discovered for every different pesticide. The RSD for all these pesticides is 1.155, 1.81, and 2.09, LOD is 3.81, 5.973, 6.897 mmol L^{-1}, LOQ is 11.55, 18.8, and 20.9 mmol L^{-1} respectively. The LR are 2.6 and 20 mmol L^{-1}. The pesticide residues found are accurate.

5. Acknowledgment

The authors would like to thank the Acharya Narendra Dev College, University of Delhi, New Delhi, India, and the authors declare that they have no competing interests

6. References

[1] R. Kaur, G. K. Mavi, S. Raghav, I. Khan, Pesticides classification and its impact on environment, Int. J. Curr. Microbiol. Appl. Sci., 8 (2019) 1889-1897. https://doi.org/10.20546/ijcmas.2019.803.224

[2] V. Rathee, A. Kumar Dubey, M. Kaur, Effects of pesticides on human health, J. Forensic Sci. Res., 7 (2023) 034-039. https://doi.org/10.29328/journal.jfsr.1001047

[3] B. Hicks, Agricultural pesticides and human health, geology, and human health, teach the earth, 2012. https://serc.carleton.edu/NAGTWorkshops/health/case_studies/pesticides.html

[4] V. Philippe, A. Neveen, A. Marwa, A. Y. A. Basel, Occurrence of pesticide residues in fruits and vegetables for the Eastern Mediterranean Region and potential impact on public health, Food Control, 119 (2021) 107457. https://doi.org/10.1016/j.foodcont.2020.107457

[5] H. D. O. Gomes, J. M. C. Menezes, J. G. M. da Costa, H. D. M. Coutinho, R. N. P. Teixeira, R. F. do Nascimento, A socio-environmental perspective on pesticide use and food production, Ecotoxicol. Environ. safe., 197 (2020) 110627. https://doi.org/10.1016/j.ecoenv.2020.110627

[6] D. Kumari, S. John, Health risk assessment of pesticide residues in fruits and vegetables from farms and markets of Western Indian Himalayan region, Chemosphere, 224 (2019) 162-167. https://doi.org/10.1016/j.chemosphere.2019.02.091

[7] A. Verdian, Apta-nanosensors for detection and quantitative determination of acetamiprid–A pesticide residue in food and environment, Talanta, 176 (2018) 456-464. https://doi.org/10.1016/j.talanta.2017.08.070

[8] B.S. Aradhana, R. Aiswarya, K.G. Praveena, M. Joshy, B.S.Reshmi, Quality and pesticides detection in fruits and vegetables, J. Emerg. Technol. Innov. Res., 8 (2021) g227-g230. https://www.jetir.org/papers/JETIR2105829.pdf

[9] V. V. Sarbhukan, L. Ragha, Establishing secure routing path using trust to enhance security in MANET, Wirel. Pers. Commun., 110 (2020) 245-255. https://doi.org/10.1007/s11277-019-06724-0

[10] C. Pundir, A. Malik, Bio-sensing of organophosphorus pesticides: A review, Biosens. Bioelectron., 140 (2019) 111348.

https://doi.org/10.1016/j.bios.2019.111348

[11] D. Wang, P. Wang, D. Liu, Z. Zhou, Fluorometric atrazine assay based on the use of nitrogen-doped graphene quantum dots and on inhibition of the activity of tyrosinase, Microchim. Acta, 186 (2019) 1-7. https://doi.org/10.1007/s00604-019-3648-6

[12] S. Muehlwald, N. Buchner, L. Kroh, Investigating the causes of low detectability of pesticides in fruits and vegetables analysed by high-performance liquid chromatography–Time-of-flight, J. Chromatogr. A, 1542 (2018) 37-49. https://doi.org/10.1016/j.chroma.2018.02.011

[13] K. Madej, T. K. Kalenik, W. Piekoszewski, Sample preparation and determination of pesticides in fat-containing foods, Food Chem., 269 (2018) 527-541. https://doi.org/10.1016/j.foodchem.2018.07.007

[14] A. Samsidar, S. Siddiquee, S. M. Shaarani, A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs, Trend. Food Sci. Technol., 71 (2018) 188-201. https://doi.org/10.1016/j.tifs.2017.11.011

[15] M. V. Navarro, M. A. Cabezon, P. C. Damiani, Simultaneous determination of pesticides in fruits by using second-order fluorescence data resolved by unfolded partial least-squares coupled to residual bilinearization, J. Chem., 2018 (2018) 217465. https://doi.org/10.1155/2018/3217465

[16] S. Narenderan, S. Meyyanathan, B. Babu, Review of pesticide residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques, Food Res. Int., 133 (2020) 109141. https://doi.org/10.1016/j.foodres.2020.109141

[17] Y. V. Plekhanova, A. Reshetilov, Microbial Biosensors for the determination of pesticides, J. Anal. Chem., 74 (2019) 1159-1173. https://doi.org/10.1134/S1061934819120098

[18] A. Mishra, J. Kumar, J. S. Melo, B. P. Sandaka, Progressive development in biosensors for detection of dichlorvos pesticide: A review, J. Environ. Chem. Eng., 9 (2021) 105067. https://doi.org/10.1016/j.jece.2021.105067

[19] E. N. Esimbekova, V. P. Kalyabina, K. V. Kopylova, I. G. Torgashina, V. A. Kratasyuk, Design of bioluminescent biosensors for assessing contamination of complex matrices, Talanta, 233 (2021) 122509. https://doi.org/10.1016/j.talanta.2021.122509

[20] B. Rajangam, D. K. Daniel, A. I. Krastanov, Progress in enzyme inhibition based detection of pesticides, Eng. Life Sci., 18 (2018) 4-19. https://doi.org/10.1002/elsc.201700028

[21] H. Patel, D. Rawtani, Y. Agrawal, A newly emerging trend of chitosan-based sensing platform for the organophosphate pesticide detection using Acetylcholinesterase-a review, Trend. food Sci. Technol., 85 (2019) 78-91. https://doi.org/10.1016/j.tifs.2019.01.007

[22] N. S. Sulaiman, K. Rovina, V. M. Joseph, Classification, extraction and current analytical approaches for detection of pesticides in various food products, J. Consum. Prot. Food Safe., 14 (2019) 209-221. https://doi.org/10.1007/s00003-019-01242-4

[23] M. Fuyal, B. Giri, A Combined system of Ppaper device and portable spectrometer for the detection of pesticide residues, Food Anal. Method., 13 (2020) 1492-1502. https://doi.org/10.1007/s12161-020-01770-y

[24] N. Kalyani, S. Goel, S. Jaiswal, On-site sensing of pesticides using point-of-care biosensors: a review, Environ.l Chem. Lett., 19 (2021) 345-354. https://doi.org/10.1007/s10311-020-01070-1

[25] L. Karadurmus, S. Kaya, S. A. Ozkan, Recent advances of enzyme biosensors for pesticide detection in foods, J. Food Meas. Charact., 15 (2021) 4582-4595. https://doi.org/10.1007/s11694-021-01032-3

[26] M. Arjomandi, H. Shirkhanloo, A review: analytical methods for heavy metals determination in environment and human samples, Anal. Methods Environ. Chem. J., 2 (2019) 97-126. https://doi.org/10.24200/amecj.v2.i03.73

[27] S. Teimoori, H. Shirkhanloo, A. H. Hassani, M. Panahi, N. Mansouri, An immobilization of aminopropyl trimethoxysilane-phenanthrene carbaldehyde on graphene oxide for toluene extraction and separation in water samples, Chemosphere, 316 (2023) 137800. https://doi.org/10.1016/j.chemosphere.2023.137800

[28] S. Teimoori, H. Shirkhanloo, A. H. Hassani, M. Panahi, N. Mansouri, Rapid extraction of BTEX in water and milk samples based on functionalized multi-walled carbon nanotubes by dispersive homogenized-micro-solid phase extraction, Food Chem., 421(2023) 136229. https://doi.org/10.1016/j.foodchem.2023.136229

[29] S. Teimoori, H. Shirkhanloo, A. Hassani, M. Panahi, and N. Mansouri, New extraction of toluene from water samples based on nano-carbon structure before determination by gas chromatography, Int. J. Environ. Sci. Technol., 20 (2023) 6589-6608. https://doi.org/10.1007/s13762-023-04906-9

[30] R. Ashouri, H. Shirkhanloo, A. Rashidi, S. Mirzahosseini, N. Mansouri, Dynamic and static removal of benzene from air based on task-specific ionic liquid coated on MWCNTs by sorbent tube-headspace solid-phase extraction procedure, Int. J. Environ. Sci. Technol., 18 (2021) 2377-2390. https://doi.org/10.1007/s13762-020-02995-4

[31] J. Rakhtshah, H. Shirkhanloo, N. Esmaeili, A rapid extraction of toxic styrene from water and wastewater samples based on hydroxyethyl methylimidazolium tetrafluoroborate immobilized on MWCNTs by ultra-assisted dispersive cyclic conjugation-micro-solid phase extraction, Microchem. J., 170 (2021) 106759. https://doi.org/10.1016/j.microc.2021.106759

[32] O. V. Omotoyinbo, E. O. Awojulu, D. M. Sanni, Phytochemical screening, antioxidant and tyrosinase inhibitory studies of methanol leaf extracts of two tomato varieties, Highligh. BioSci., 3 (2020) 20216. https://doi.org/10.36462/H.BioSci.20216

[33] P. Angelini, R. Venanzoni, G. Angeles Flores, B. Tirillini, G. Orlando, L. Recinella, Evaluation of antioxidant, antimicrobial and tyrosinase inhibitory activities of extracts from Tricholosporum goniospermum, an edible wild mushroom, Antibiotics, 9 (2020) 513. https://doi.org/10.3390/antibiotics9080513

[34] T. J. Al-Khafaji, F. Wong, P. S. Fleming, N. Karpukhina, R. Hill, Novel fluoride and strontium-containing bioactive glasses for dental varnishes-design and bioactivity in Tris buffer solution, J. Non-Crystalline Solids, 503 (2019) 120-130. https://doi.org/10.1016/j.jnoncrysol.2018.09.037

[35] P. Pushankina, G. Andreev, I. Petriev, Hydrogen permeability of composite Pd–Au/Pd–Cu membranes and methods for their preparation, Membranes, 13 (2023) 649. https://doi.org/10.3390/membranes13070649

[36] A. K. Singh, I. Tiwari, Nanomaterial synthesis and mechanism for enzyme immobilization: part II, Nanomaterials in biofuels research book, Springer publisher, pages191-212, 2020. https://doi.org/10.1007/978-981-13-9333-4_8

[37] S. Aggarwal, A. Chakravarty, S. Ikram, A comprehensive review on incredible renewable carriers as promising platforms for enzyme immobilization and thereof strategies, Int. J. Biol. Macromol., 167 (2021) 962-986. https://doi.org/10.1016/j.ijbiomac.2020.11.052

[38] T. Rasheed, Carbon dots as robust class of sustainable and environment friendlier nano/optical sensors for pesticide recognition from wastewater, TrAC Trend. Anal. Chem., 160 (2023) 116957. https://doi.org/10.1016/j.trac.2023.116957

[39] T. Ban, M. Guo, Y. Wang, J. Ma, X. Wang, Z. Wang, Efficient and durable vanadium flow batteries enabled by high-performance fluorinated poly (aryl piperidinium) membranes, J. Mater. Chem. A, 11 (2023) 24013-24025. https://doi.org/10.1039/D3TA05382B