College of Natural and Computational Sciences School of Post Graduate Studies

Department of Microbial, Cellular and Molecular Biology

Isolation and Characterization of Dichlorodiphenyltrichloroethane (DDT) Degrading Fungi Isolated from Agro-Industrial Effluent and Farm Soils

A Thesis Submitted to the School of Post Graduate Studies of Addis Ababa University in Partial Fulfillment of the requirement for the Degree of Masters of Sciences (M.Sc.) in Applied Microbiology

By: **Girma Ebsa Bedasa**

Advisor: Tesfaye Alemu (Ph.D.)

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DECLARATION

Girma Ebsa Bedasa hereby declare that “Isolation and Characterization for Dichlorodiphenyltrichloroethane (DDT) Degrading Fungi Isolated from Agro-Industrial Effluent and Farm Soils” is my own work and it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University except where due acknowledgement has been made in the text. It is submitted for the degree of Master of Science in Applied Microbiology to Addis Ababa University College of Natural and Computational Sciences School of Post Graduate Studies.

Candidate	**Signature**	**Date**
Girma Ebsa	----------	----------

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As thesis research Advisor, we here certify that the thesis prepared by Girma Ebsa which were under our guidance throughout the research work, entitled "**Isolation and Characterization for Dichlorodiphenyltrichloroethane (DDT) Degrading Fungi Isolated from Agro-Industrial Effluent and Farm Soils**" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology fulfills with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Dr. Tesfaye Alemu (Advisor)	_____	_____.
Dr. Asnake Desalegn (Co-Advisor)	_____	_____.
Dr. Mesele Admassie (Co-Advisor)	_____	_____.
Ms. Birhanu Gizaw (Co-Advisor)	_____	_____.
Prof. Fasil Asefa (External Examiner)	_____	_____.
Dr. Dagim Jirata (Internal Examiner)	_____	_____.
Dr. Helen Nigussie (Chair of the Dep.)	_____	_____.

DEDICATION

I want to dedicate this MSc thesis to my older brother Adera Ebsa, who lit the candle of knowledge in my life when I started my education in grade 1 until my entire course study.

ACRONYMS

AAU	Addis Ababa University
ANOVA	Analysis of Variance
AOPs	Coagulation and Advanced Oxidation Processes
ASD	Autism Spectrum Disorders
BBD	Box-Behnken Design
CDC	Center for Diseases Control
CEC	Cation Exchange Capacity
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DW	Dry Weights
Eh	Redox Potential
FAO	Food and Agricultural Organization
GC-ECD	Gas Chromatograph-Electron Capture Detector
GMOs	Genetically Modified and Engineered Organisms
HPLC	High Power Liquid Chromatography
HRP	Horseradish Peroxidase
LPCB	Lactophenol Cotton Blue
MALDI TOF-MS Mass Spectrometry	Matrix-Assisted Laser Desorption, Ionization, and Time of Flight

MnP	Manganese Peroxidases
MRL	Maximum Residue Limit
MSM	Minimal Salt Medium
NF	Nanofiltration
PDA	Potato Dextrose Agar
PPEs	Personal Protective Equipments
RPM/min	Rotation per minute
RSM	Response Surface Methodology
Sp. /*spp.*	*Spp*ecies
USEPA	Environmental Protection Agency
TI	Tolerance Index
VOCs	Volatile Organic Compounds
WHO	World Health Organization
WRF	White-Rot Fungus

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is an environmentally hazardous synthetic compound that is resistant to usage. Despite being illegal in most countries, it is used as a pesticide to fight malaria in most malarial zone part of Ethiopia. The main objective of this study is to screen, characterize, and evaluate potential DDT-degrading fungi and their synergetic interaction effects for mycoremediation purposes. The composite of 150 soil and effluent samples was collected from Addis Ababa and East Shewa Zone, mainly Ziway and Koka. Fungal isolation and screening were performed using a serial dilution on potato dextrose agar growth media. MALDI-TOF MS technology was used for fungal identification. Fungal biomass production and sporulation capacity were examined and optimized using a Box-Behnken experimental design. The potential DDT-tolerant fungi were studied based on growth factor optimization. Gas Chromatograph-Electron Capture Detector technology was used for the DDT degradation study. Fungal identification results revealed that the finally selected isolates, AS1 and T1, were *Asppergillus niger* and *Trichoderma koningii*, respectively. The optimization results confirmed that the co-inoculated isolates AS1T1 had a maximum biomass (1.01±0.16 g) and spore count (5.74±0.37 log spore/mL) and were selected as possible DDT-degrading fungi. The GC-ECD result analysis revealed that fungal-co-cultured *A. niger* and *T. koningii* in DDT-amended liquid medium were able to degrade DDT into its metabolites Dichlorodiphenyldichloroethane (DDD) and Dichlorodiphenyldichloroethylene (DDE). The results also revealed that 99.5–99.99% of DDT and its metabolites degraded from initial concentrations of 1750, 3500, 5250, and 7000 ppm. The co-inoculated fungi *A. niger* and *T. koningii* are promising candidates for the removal of DDT and its metabolites from polluted environments.

Keywords: Dichlorodiphenyldichloroethane, Dichlorodiphenyldichloroethylene, Dichlorodiphenyltrichloroethane, Degradation, Gas Chromatography-Electron Captured Detector, Matrix-Assisted Laser Desorption, Ionization, Optimization

1. INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) is a persistent organochlorine synthetic chemical used on a global scale to control agricultural pests and the spread of diseases caused by pests (Kolan and Hall, 2023). Although DDT chemicals were used to control agricultural productivity, concerns have been raised about the environmental dangers caused to both humans and wildlife. The threats to human health from DDT exposure are both acute and chronic, including endocrine disrupters, decreased intelligence, reproductive abnormalities, probable carcinogens, mutagenic, and cause teratogenicity (Yang *et al*., 2006; Mrema *et al*., 2013; Encarnação *et al*., 2019).

DDT and its residues are considered dangerous due to their low solubility in water and high solubility in organic solvents, with their persistency, bioaccumulation, toxicity, and long-distance pollutant transport characteristics (Tasselli *et al*., 2023; Xiao *et al*., 2023). Another problem is the conversion of DDT into its obsolete form, which can possibly have more negative effects than the formal DDT (Riyaz *et al*., 2023). The persistence of DDT in the soil (half-life of 2 to 15 years) and water bodies (half-life of 150 years) is because of the absence of innate microbial systems that have DDT-degrading enzymes (Satish *et al*., 2017; Cui *et al*., 2022; Porwal, 2023). DDT residues are still constantly detected in soil and foodstuffs; human and animal samples are a major problem (Bolor *et al*., 2018; Rajput *et al*., 2022; Rose, 2023). For instance, in Zhejiang Province, China, the detection rate of DDT and its metabolites in soil samples ranged from 0.007 to 1.208 mg/kg, which exceeded the second-level Chinese soil environmental quality standard for farmland soil (Cui *et al*., 2022). In Ethiopia, a study by Kassegne *et al*. (2020) showed that the mean DDT residue detected in the samples taken from Aba Samuel reservoir and Akaki River in central Ethiopia, Addis Ababa, was 112.56 µg/kg and 11.39 ngl-1, where vegetable farms and solid waste dumps exist and exceed the FAO/WHO-set maximum residue limit (MRL = 0.01 mg/kg).

Bioremediation is one of the most novel and cost-effective ways of decontaminating and detoxifying DDT from solid sludge, soil, sediments, groundwater pollution, and the whole environment (Pradhan *et al*., 2023; Tonello *et al*., 2023; Reddy *et al*., 2024). Bioremediation converts DDT entirely into CO_2 and H_2O without the accumulation of intermediates (Dar *et al*., 2023; Panwar and Mathur, 2023; Wang *et al*., 2023). Degradation of DDT is also dependent on a

wide variety of environmental parameters such as temperature, pH, water potential, initial concentration of DDT, and available nutrients (Xie *et al*., 2011; Thirumoorthy *et al*., 2023).

Currently the degradation of environmental DDT pollutants is promoted by the biorestoration of naturally occurring microbes using biostimulation techniques, and bioaugmentation technology for DDT pesticide-degrading microflora is encouraged (Mawang *et al*., 2021; Porwal, 2023; Reddy *et al*., 2024). DDT residues biodegrade slowly in soil and water. The most recent study was done on microbial DDT degradation in a very small amount of DDT concentration up to 100 ppm (Al-Rashed, 2021), and there is a limited study in high concentrations on DDT bioremediation. As far as reviewed different literature, most research conducted in Ethiopia focused on DDT residual analysis; however, there is a scarce study on DDT bioremediation.

DDT-degrading fungi have been extensively reported, including WRF (*T. versicolor, P. chrysospporium, and Pleutorus*, *Pleutorus ostreatus*), brown-rot (*Gloeophyllum trabeum, Fomitopsis pinicola, and Daedalea* and *Daedalea dickinsii*), and other filamentous fungi (*Penicillium citrinum, Asppergillus fumigatus, Asppergillus terreus, and Trichoderma harzianum*) (Purnomo *et al*., 2008; Satish *et al*., 2017; Mori *et al*., 2018; Bisht *et al*., 2019). The co-culture-mediated mycoremediation approach is proposed as an alternative solution for removing DDT from the environment. A statistically designed BBD-RSM has been proposed to optimize the conditions for the effective biodegradation of DDT pesticides assay (Okçu *et al*., 2018; Blachnio *et al*., 2023; Krishnani *et al*., 2023).

Fungi have the ability to adapt to new environmental changes and chemical stressors by generating and modifying metabolic patterns that help them take a competitive advantage over others (Jansson *et al*., 2023; Vaksmaa *et al*., 2023). Fungal *spp*ecies have a significant functional role in soil remediation and are good recyclers of harmful organic chemicals into safe products (Ceci *et al*., 2019; Singh *et al*., 2021a; Singh *et al*., 2021b). Fungi use their inherent specificities, such as enzymes, to degrade DDT more rapidly and convert it into less toxic substances (Tortella *et al*., 2005; Gianfreda *et al*., 2016; Ijoma and Tekere, 2017). Fungi and fungi mostly join in consubstantial ecology and interact with each other, as this interaction is important to achieve synergism and competition within varying microorganisms (Leskovac and Petrović, 2023). The

combined and inductive actions of several enzymes have recently been confirmed to increase the potential for degradation in a variety of consortia.

Few studies have been done on the ability of co-culture of fungi to increase the degradation of DDT pollutants (Leskovac and Petrović, 2023). The present study aimed to investigate the potential DDT-degrading fungi isolated from agro-industrial effluent and farm soil, identify the isolates that have synergetic interaction effects, carry out DDT degradation sequentially, optimize growth factors, and formulate bioinoculants for mycorrhizal purposes.

1.1. Objective of the study

1.2. General Objective

The general objectives of this study were to isolate, screen, characterize, and optimize the potential DDT and its residual degrading fungi for their synergistic interaction.

1.3.The Specific Objectives were to:

1. Isolation, screening, and characterization of the potential DDT-degrading fungi from agroindustrial waste
2. Evaluating the effectiveness of the isolates to degrade DDT
3. Optimization of DDT-degrading selected fungi using response surface methodology based on the Box-Behnken design

1.4.Hypotheses

I). H_A: There are appropriate methods for isolating, screening, characterizing, and optimizing potential DDT-degrading fungi from polluted environments.
II). H_A: There is potential synergistic interaction of DDT-degrading fungi isolated from agro-industrial polluted environments.
III). H_A: Fungi have a potential role in the mycoremediation process of DDT.

1.5.Statement of the Problem

Currently around 1.8 billion people engage in the agricultural sector globally. In Ethiopia, agriculture is the backbone of the economy, and at the present time, above 80% of the total population is directly involved in agricultural sectors (Alemu *et al.*, 2003; Ashine *et al.*, 2024). In order to advance the productivity of the agricultural sector, a significant number of agrochemicals, especially pesticides, including DDT insecticide, fungicide, and herbicide, had been used (Chala, 2022). DDT is also used to protect against the spread of various human diseases caused by malaria. Even if DDT increases agricultural productivity and controls insect-borne diseases, concerns have been raised concerning the environmental dangers to both humans and wildlife (Li *et al.*, 2024).

These practices increase the amount of non-degradable DDT pesticide residue in the environment. This may lead to environmental pollution and affect both human health and wildlife because DDT and its residue are consumed with food without any sort of treatment (Sawant et *al*., 2022). Children, pregnant women, and all other groups are severely harmed by high DDT ingestion levels (Gumovskaya *et al*., 2023). People employed in fields for crop harvest and spraying for control of pests, as well as consumers of such food, may greatly suffer from DDT pesticides-derived diseases (Maja, 2022).

DDT has been linked to a wide range of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disorder (Islam *et al*., 2022). Acute risks associated with nerve and eye irritation and damage, headaches, faintness, nausea, fatigue, and systemic poisoning sometimes become theatrical and even occasionally fatal (Donham and Thelin, 2016). The chronic health outcomes of DDT may occur for years after even minimal exposure to DDT pesticides in the environment or result from the DDT pesticide residues that we ingest through our food and water (Sharma *et al*., 2020). It also increases the risk factor for autism spectrum disorders (ASD) for children born to mothers exposed to organochlorine pesticides, DDT (Xu *et al*., 2023). DDT residues biodegrade slowly in soil and water. Yet, there is little research on DDT bioremediation at high concentrations, and the most recent work focused on microbial DDT breakdown at very low DDT concentrations up to 100 ppm (Al-Rashed, 2021). As far as reviewed different literature, most research conducted in Ethiopia focused on DDT residual analysis; however, there is a scarce study on DDT bioremediation using fungi. This study was undertaken on potential DDT and its residual degrading fungi isolated from agro-industrial effluent and farm soil to identify the isolates that can carry out DDT degradation, optimize growth factors, and formulate bioinoculants for mycoremedial purposes.

2. LITERATURE REVIEW

2.1. Historical Application and Utilization of DDT

Organochlorine insecticide DDT is white in color, volatile, tasteless, and nearly odorless (Li *et al*., 2023). DDT is a synthetic chemical that is produced via a sulfuric acid-catalyzed reaction between chloral and chlorobenzene (de Andrade *et al*., 2023). DDT was first discovered in 1874 by Zeidler (Forbes *et al*., 2021; Rizqi *et al*., 2023). DDT is cheap to produce, much like other organochlorine pesticides. DDT contains two main metabolites, p,p'-DDE or DDE and p,p'-DDD or DDD, as well as two isomers, p,p'-DDT and o,p'-DDT (Forbes *et al*., 2021; Mukasa *et al*., 2022).

DDE and DDD are two of the metabolites that are typically present in organisms and sediments, respectively (El-Mabrok, 2022). The United States Environmental Protection Agency states that the insecticidal effects of DDT were first discovered in 1939 (USEPA, 1975). In 1942 and 1943, the Department of Agriculture's laboratory in Orlando, Florida, did more testing on the insecticide in the United States (Tissot *et al*., 1954). These tests confirmed that DDT was effective in reducing the spread of vector disease. The USA military utilized DDT for the first time in World War II to combat typhus, body lice, malaria, and bubonic plague and used it for insecticides until the late 1970s, until it was banned in the majority of developed nations. Since 1940, the insecticide has been widely employed to control agricultural pests (Metcalf, 1980; Clarke and Lean, 2022).

Ethiopia has historically utilized few chemical pesticides, but as a result of increased food production from agricultural activities and the growth of the floriculture business, chemical pesticide use has increased (Tesema *et al*., 2022). The use of pesticides in Africa accounts for about 2–4% of the $31 billion global pesticide market (Ray et al., 2024).

Organochlorines are chemical compounds made up of chlorine, carbon, hydrogen, and sometimes additional atoms. Organochlorine pesticides include DDT (Bose *et al*., 2021; Rajak *et al*., 2023). Before the US Environmental Protection Agency (EPA) banned its usage in 1972, it was widely employed in the country to control insects (Giroux *et al*., 2024). DDT is still used in certain African nations, such as Ethiopia, where malaria vector control is crucial, even if it were illegal (Sadasivaiah *et al*., 2007). The application of DDT led to the successful eradication of the malaria vector (Abbasi *et al*., 2022).

Since the 1960s, chemical pesticides have been used in Ethiopia to develop the growth of commercial farms (Rao and Morimoto, 2020) and increased in the early 2000s as a result of horticulture development (Wm-Bekele *et al*., 2024). Farmers are led to believe that using pesticides like DDT is the only option to prevent crop losses by organizations that advocate the sale of pesticides (Hashemi and Damalas, 2010). In addition to importing about 3,800 tons of pesticides every year, Ethiopia also receives pesticides by donation (Loha *et al*., 2018). Approximately 72% of the pesticides imported are insecticides, 25% are herbicides, 2.6% are fungicides, and 1.3% are miscellaneous products like disinfectants and rodenticides.

In the late 1990s, the nation legalized pesticide registration and established a monitoring system (Castro-Vargas and Werner, 2023). Even though there are regulations governing pesticide registration, they are not effectively enforced when it comes to pesticide importation, testing, and application (Mengistie, 2016; Teshome *et al*., 2023). Therefore, it is common to find a lot of prohibited pesticides in Ethiopia and use them illegally (Habtamu *et al*., 2023). When personal protection equipment (PPE) is not used, improper techniques of application can occur. This leads to the uncontrolled release of DDT pesticides into the environment, hence exposing people to DDT (Usip *et al*., 2023; Barrón *et al*., 2024).

2.2. DDT Impact on the Environment

DDT is incredibly persistent in the environment; unfortunately, up to 80% of the DDT used could leak into the environment within a few months, endangering both the ecosystem and human health (Parra-Arroyo *et al*., 2022). DDT has a half-life of 20-30 years in soil (Cui *et al*., 2024). In an aquatic environment, DDT has a half-life of almost 150 years (Wolfe *et al*., 1977). Following its application on fields, DDT is carried by rainfall into subterranean streams or other ecological water sources (Pradhan *et al*., 2022). Although the amount of DDT in the water may not be very high, aquatic life, particularly zooplankton, absorbs it and raises its concentrations to extremely high levels (Dhanaraj, 2024). The amount of DDT in the food chain gradually increases. The fish that consume zooplankton are more concentrated, and the *spp*ecies most harmed by DDT are birds. DDE has produced major environmental issues all over the world and is more persistent in the environment than DDT. Tragically, dichlorodiphenyldichloroethylene, a very persistent, physiologically active metabolite of DDT with antiandrogenic qualities, and the compound's

environmental persistence have contributed to the problem of DDT in the environment (Umulisa *et al*., 2020; Ebsa *et al*., 2021).

2.3. DDT Impacts on Human Health and Animals

Public awareness of DDT's effects would not reach its zenith until Rachel Carson's groundbreaking book Silent Spring (Rachel, 1962; Sullivan, 2004). The development of contemporary environmental toxicology and chemistry can be attributed to studies that showed how DDT affected wildlife populations (Matthiessen *et al*., 2018; Rezende-Teixeira *et al*., 2022). The impact of DDT on human health at low ambient concentrations is uncertain (Wang *et al*., 2021). Human symptoms with high dosage exposure can include nausea, convulsions, tremors or instability, and trembling.

Studies conducted on lab animals have demonstrated DDT effects on the liver and reproductive system. It is thought that DDT may cause cancer in people (Douglas and Douglas, 2019). DDT's effects on the environment and human health are dependent upon exposure duration, frequency, and dosage (Cavalier *et al*., 2023; Meftaul *et al*., 2023). Its effects are also contingent upon an individual's health and certain environmental circumstances (WHO, 1990). DDT residue is found in practically every human body (Miao *et al*., 2023).

There are three ways that humans can be exposed to DDT: during pregnancy, when nursing, and when they consume contaminated food that contains DDT. People are mostly exposed to DDT through their diet. Consuming various foods is responsible for 90% of the DDT residue that is stored in the human body (Barau *et al*., 2023). There is DDT contamination in a number of common foods. Another potential source of human exposure to pesticides is the indoor residual spraying (IRS) method for vector control (Pryce *et al*., 2022). The amount of food ingested and the concentration of DDT and its metabolites in the food may determine the dietary exposure (Derouiche *et al*., 2023).

The metabolic system of infants is not developed enough to eliminate DDT compared to adults; that is why they are more vulnerable to the harmful effects of pesticides when they are breastfed (Mekonen *et al*., 2021; Qi *et al*., 2022). Infants consume more food relative to their body weight (Zheng *et al*., 2024). DDT exposure during pregnancy may have an impact on an infant's

neurodevelopmental growth between the ages of one and six months (Mérida-Ortega *et al*., 2019). According to an Indian and Pakistan study, some infants' breast milk contained concentrations of total DDT over the daily allowable dosage, which poses a health risk (Mehta *et al*., 2020; Sana *et al*., 2021).

When it comes to balancing the food given to infants over the age of six months, maize is the most common grain in Ethiopia (Abeshu *et al*., 2016). Farmers utilize DDT, which is prohibited for use in agriculture, to control these insects. DDT was discovered in maize samples taken from the Jimma zone above the Maximum Residue Limit (MRL) in southwest Ethiopia (Mekonen *et al*., 2015; Mekonen *et al*., 2021). There may be health problems for infants who eat maize polluted with DDT and its metabolites.

An essential first step in regulating the use of DDT pesticides on food crops is consumer risk assessment (Fenner-Cri*spp*, 2010; Akpan *et al*., 2023). Despite all of these issues, no research has been done to quantify the hazards and exposure of newborns to pesticides, namely DDT, in Ethiopia (Tadele and Amado, 2023).

It is rapidly absorbed through an insect's outer coating but is poorly absorbed through the skin of mammals (Sousa *et al*., 2020). Laboratory animals exposed to DDT have convulsions, tremors, hyper excitability, and impaired coordination (Naik *et al*., 2024; Smith, 2024). DDT is categorized as a B2 carcinogen by the EPA (Rusiecki *et al*., 2024).

DDT has been shown to cause cancer in experimental animals; there is either insufficient or no evidence linking it to cancer in people (Huff, 1993; Calle *et al*., 2002). No cancer was discovered in 19 years of research involving workers at a DDT production site (WHO, 1979). However, according to a recent study conducted by Wan *et al*. (2022), there was an increased incidence of breast cancer and positive tumors in girls whose mothers had been exposed to high doses of DDT throughout their pregnancies. According to WHO. (1979), animals exposed to DDT over an extended period of time experience alterations in their livers and lesions in their livers at potentially deadly levels. The body breaks down DDT into a number of breakdown products known as metabolites, including DDE. There is an accumulation of DDT and DDE in the body's fatty tissues. The amount of DDT and its metabolite DDE that has entered people's bodies has been estimated

by CDC experts based on measurements of these chemicals' presence in serum. Only a tiny percentage of people have detectable DDT. The majority of people had noticeable DDE. DDE is a sign of prior exposure and remains in the body longer than DDT (Beard *et al*., 2006; CDC 2009).

2.4. Water and Soil Treatment Techniques for DDT Pesticides Removal

Many developing nations continue to manufacture and utilize DDT, which accumulates in the soil and waterways. Even with the signing of the Stockholm Convention, which outlawed the use of pesticides, the impacted countries stopped manufacturing the DDT chemicals (Mohapatra, 2021). The contaminated sites in Europe are approximately between 300.000 to 1.5 million and the estimated total costs for the remediation of the sites in Europe are estimated between 59 and 109 billion euro (Lepke, 2003). It is inevitable to apply cost-effective remediation processes in cleaning up the pesticides, such as DDT. There is a need for investigating possible DDT remediation techniques. Both the high emergence of water and soil DDT contaminants and the challenges that face their natural breakdown have led to the development of many treatment techniques such as chemical, physical, and microbial (biological) methods (Ahmed *et al*., 2021).

2.4.1. Chemical DDT Treatment Techniques

DDT pollutes soil and water ecosystems. Chemical treatment entails a range of chemical reactions that facilitate the hydrolysis of dangerous DDT pollutants into less hazardous compounds. Coagulation and advanced oxidation processes (AOPs), such as zonation and Fenton treatment, are the principal chemical techniques (Ateş, 2019). Chemical DDT treatments are usually used in combination with photo-catalysis and/or membrane techniques (Brovini *et al*., 2023). Even though chemical DDT treatment techniques are destructive and rapid, they are very costly, have variable effectiveness, and are complex (Kamalesh *et al*., 2023; Mandal *et al*., 2024).

2.4.2. Physical DDT Treatment Techniques

Membrane filtering technology (MF) is heavily utilized in DDT-contaminated water treatment processes. As long as the target contaminants and membrane types are known, filtration can be used at any stage of the water treatment process. The type of filtering depends on the membrane cut-off size (Obotey and Rathilal, 2020). For the removal of large-sized organic compounds, such

as DDT, from water, nanofiltration membranes with a pore size of 0.4 µm are appropriate. The key procedures that control the removal of DDT by MF are adsorption on the membrane and repulsion (steric and electrostatic) of the membrane (Pang *et al*., 2010; Khoo *et al*., 2022). The World Health Organization (WHO) has set a maximum residual limit for DDT insecticides at 1 µg/L concentrations in water because of their public health hazard (Sang *et al*., 2022). The filtration capacity of the membranes was assessed in a laboratory-scale experiment where the amounts of DDT contaminants were measured using HPLC (Brovini *et al*., 2023).

DDT Physical treatment for soil remediation includes methods that isolate pollutants from soil particles. The process of separation lowers the volume of the DDT pollutant by moving it to another medium, such as air or water, and collecting it in a concentrated form. Therefore, physical separation treatment typically necessitates a treatment train in order to finish the process. *Ex situ* (soil excavation) or in situ physical treatment is carried out using separation technologies. The ability to restore soil without excavating or moving it is one of the primary benefits of in situ treatment. Treatment periods for in situ remediation are typically longer. It is also more challenging to guarantee treatment homogeneity due to the subsurface's variability. Because in situ treatment causes the DDT contaminants to migrate out of the contaminated soil zone, it also has to consider preventing the spread of DDT contamination.

Three types of *in situ* physical remediation of DDT-contaminated soil exist: 1) soil vapor extraction in the vadose zone and various techniques to improve contaminant removal by vaporization; 2) air sparging, which involves forcing air to bubble through groundwater to volatilize volatile organic compounds (VOCs); and 3) soil flushing, which involves using chemicals like surfactants to improve the removal efficacy of water flowing through contaminated soil in addition to the flushing action of groundwater in pump-and-treat systems (Gomes *et al*., 2013; Chattopadhyay et *al*., 2015; Chaukura *et al*., 2022).

2.4.3. Microbial DDT Treatment Techniques

DDT contaminants are removed from soil and water by microbial degradation (bioremediation), which happens naturally with the help of local soil organisms, that include micro-algae, bacteria, or fungi are typically suitable (Tarla *et al*., 2020; Raffa and Chiampo, 2021). A number of factors need to be taken into consideration when selecting the best microbial DDT degradation method.

These include the kind and availability of the pollutant's microbial populations, the types and physicochemical condition of the soil and water, the kind and condition of the microbial population in the soil, and external factors like temperature, pH, the presence of nutrients, and oxygen (Al-Rashed *et al*., 2021; Melendez-Pastor *et al*., 2023).

Bio-mixtures are generally made of humic-rich ingredients to enhance soil, microorganisms, and DDT pesticide retention (Hassaan *et al*., 2022). The microorganisms in the bio-mixture may originate from exogenous or endogenous sources. Microorganisms that are more suited to function as endogenous biodegrading microorganisms will be present in soil samples obtained from areas heavily exposed to DDT insecticides. However, some exogenous sppecies may be employed in place of others due to the biomass restrictions of some indigenous sppecies (Rabbani *et al*., 2024; Rehman *et al*., 2024). It should be noted that the toxicity of DDT insecticides to a variety of bacteria and fungi makes it difficult to degrade the chemical with microorganisms (Abioye *et al*., 2021; Sarker *et al*., 2021).

There are two types of microbial DDT treatment: anaerobic and aerobic. It is well known that aerobic treatment degrades dichlorinated pesticides, such as DDT and its main metabolites. After the aromatic ring is open, the molecule is readily broken down by typical bacterial and fungal metabolism into carbon dioxide and water. The process involves the oxidation and cleavage of the ether link as well as the hydroxylation of the chlorophenol to generate chlorocatechol (e Rocha *et al*., 2022). Reductive dehalogenation is another anaerobic method for breaking down dichlorinated insecticides without oxygen. The substance will break down into methane and carbon dioxide once the concentration of chlorine atoms is low enough (Seo *et al*., 2009; Singh and Sable, 2024). DDT pesticides have a tough time degrading microbiologically, but once a system is established, it is simple to maintain. In rare cases, pre-treatments are required. For instance, photochemical degradation of DDT pesticides or enzymatic processes may help with their biological digestion (Sharma *et al*., 2022; Abdel and Abdelhameed, 2023; Shahid *et al*., 2023).

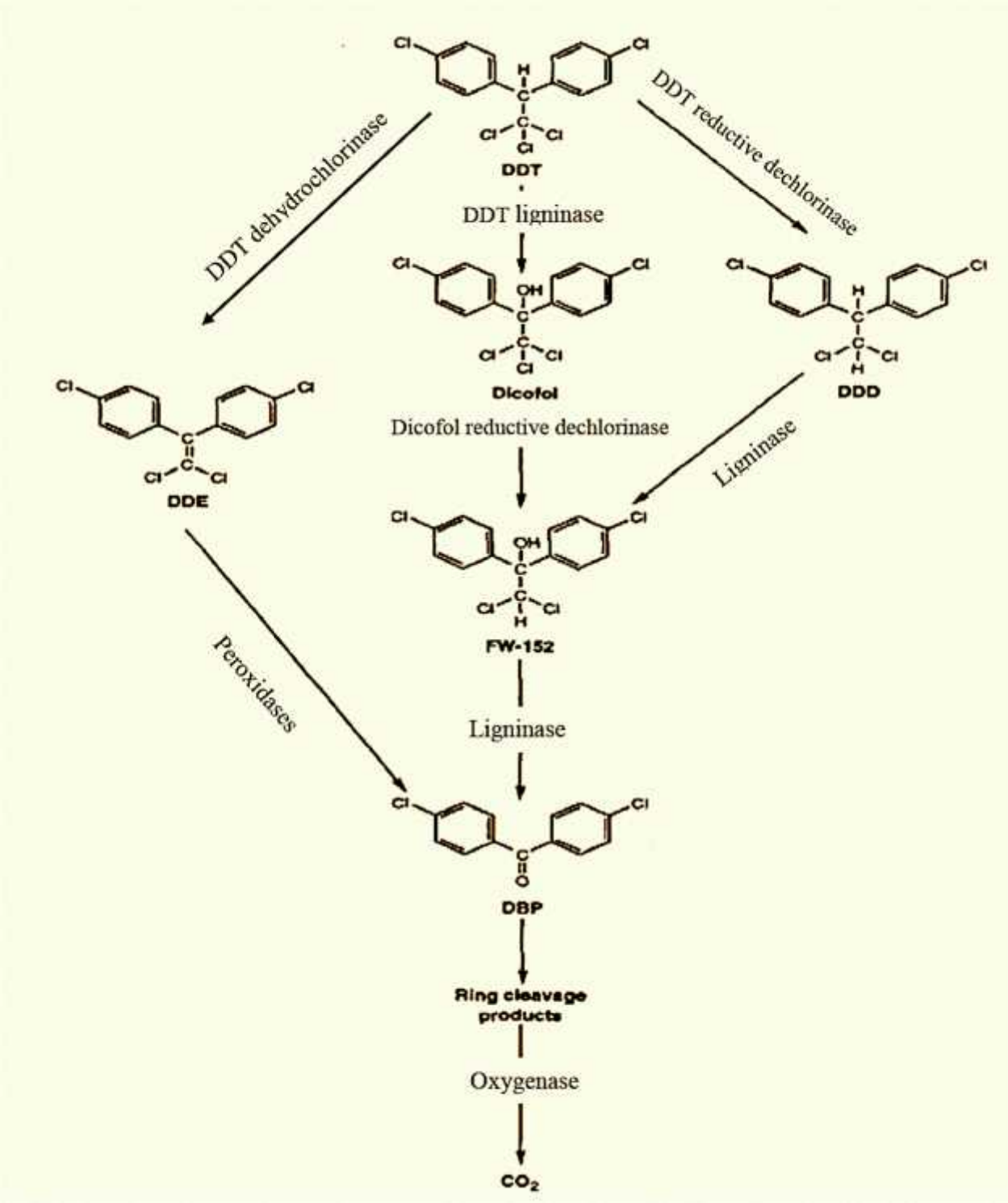


Fig. 2, Fungal mineralization of DDT Proposed pathway for DDT and its residual degradation by *Phanerochaete chrysospporium* modified from (Bumpus and Aust, 1987).

2.5. Fungal DDT Remediation

The process of converting DDT into low-molecular-weight chemicals by means of effective fungal enzymes is known as the fungal degradation of DDT pesticide (Tarfeen *et al*., 2022; Rizqi *et al*., 2023; Vijayanand *et al*., 2023). Fungal DDT remediation is an economically viable and efficient method of degrading DDT that is typically reliant on the types of fungi and environmental conditions (Kour *et al*., 2021). It is a safe and eco-friendly technique for soil and water DDT contamination remedies.

Fungi have enormous catabolic potential for the remediation of DDT pollutants and then breaking them down into less toxic compounds. They have a means of remediating DDT by secreting exudates as an energy source and cooperating together in the remediation process synergistically (Kour *et al*., 2021; Bokade *et al*., 2023). Saprophytic fungi can develop resistance, metabolizing a wide variety of organic pollutants over time (Purnomo *et al*., 2020). Many fungi *spp*ecies can play a role in DDT degradation as indicated in Table 1. White rot fungi sppecies were able to degrade seventy (70) and thirty (30) percent of DDT, res*pec*tively, within twenty-one (21) days of incubation in a low-nitrogen medium (Purnomo *et al*., 2020; Jasu *et al*., 2021).

Fungal DDT degradation primarily refers to three processes: In the first, the original parent pesticide chemical is converted into soluble and non-toxic metabolites through reduction, hydrolysis, or oxidation (Saravanan *et al*., 2021; Krithiga *et al*., 2022). These procedures require a reductive and oxidative enzyme that the fungus releases (Salem *et al*., 2023). The second step is conjugation, which increases the nature of the intermediate products' water solubility (Saravanan *et al*., 2021).Finally, peroxidases, oxygenases, and other enzymes convert DDT-intermediate metabolites into non-toxic compounds (Genuchten, 2023). Lastly, benzaldehyde is produced when lignin-degrading enzymes split the molecule's ring structure (Rani *et al*., 2023; Aggarwal and Kumari, 2024).

The combined fungal remediation technology refers to the combination of two or more different fungi *spp*ecies remediation methods to enhance the remediation of DDT pollutants and improve the remediation efficiency. Fungi-fungi interactions could lead to synergistic interaction effect. It can facilitate the degradation of DDT pollutants from diverse environmental matrices. Fungi cometabolize in mixed fungal co-cultures, potentially accelerating and eliminating DDT's harmful

effects and enhancing resistance to shifting or variable environmental conditions (Esppinosa-Ortiz *et al*., 2022).

It has been shown that a consortium of different fungi, in contrast to single strains, can degrade DDT pesticides more efficiently. Fungi-fungi remediation is the less-used combined bioremediation technology in DDT-contaminated soil and water ecosystems. It can take advantage of both different *spp*ecies of fungi. In some cases, the combined application of fungi and certain minerals has also proven more efficient for DDT pesticide removal than the application of fungi or minerals alone (Russo *et al*., 2019; Ebsa *et al*., 2024).

Table 1. Some of Fungal *spp*ecies capable of degrading DDT

Fungal *spp*ecies DDT degraders	**DDT Concentration**	**% Degradation**	**Incubation Period**	**References**
Fusarium spp	50 ppm	94	3 weeks	Kulshrestha *et al*., 2010
Penicillium spp	10 ppm	92	48 hours	Izcapa-Treviño *et al*., 2009
Trametes versicolor	709 ppb	75	15 days	Isia *et al*., 2019
Trametes versicolor	100 ppm	71	30 days	Sari *et al*., 2012
T.viride	10 ppm	98	15 days	Choudhury, 2019
Asppergillus niger	15 ppm	100	21 days	Barragan-Huerta et al., 2007
Fusarium sppecies	15 ppm	99.9	21 days	Sherif *et al*., 2014
Phlebia sppecies	100 ppm	95	7 days	Chang *et al*., 2022
Pleurotus ostreatus	5 ppm	86	7 days	Purnomo *et al*., 2017
P. chrysospporium	11.3 ppm	90	30 days	Foght *et al*., 2001

2.6. Fungal DDT Remediation Mechanisms

George Robinson used bacteria as a bioremediation technique to lessen the impact of oil *spp*ills for the first time in the 1960s. Fungal remediation mostly relies on fungi, which is called mycoremediation, to degrade DDT pollutants. Fungi in DDT-contaminated soil and water use a variety of defense mechanisms to mitigate their toxicity (Yadav and Chandra, 2020). An effective and promising method to clean up DDT-contaminated soils and water is mycoremediation, which uses fungi to detoxify DDT contaminants (Haripriyan *et al*., 2022). Fungi have great potential for the degradation of DDT pollutants, as these microorganisms can utilize DDT pollutants as sources of energy and carbon or by co-metabolizing them in the presence of other substrates (Fester. *et al.* 2014). Mycoremediation of DDT-pesticide contaminants can be grouped into five subclasses:

fungal DDT sorption, fungal DDT accumulation, fungal DDT transformation, fungal DDT mineralization, and fungal DDT degradation, as shown in Fig. 1.

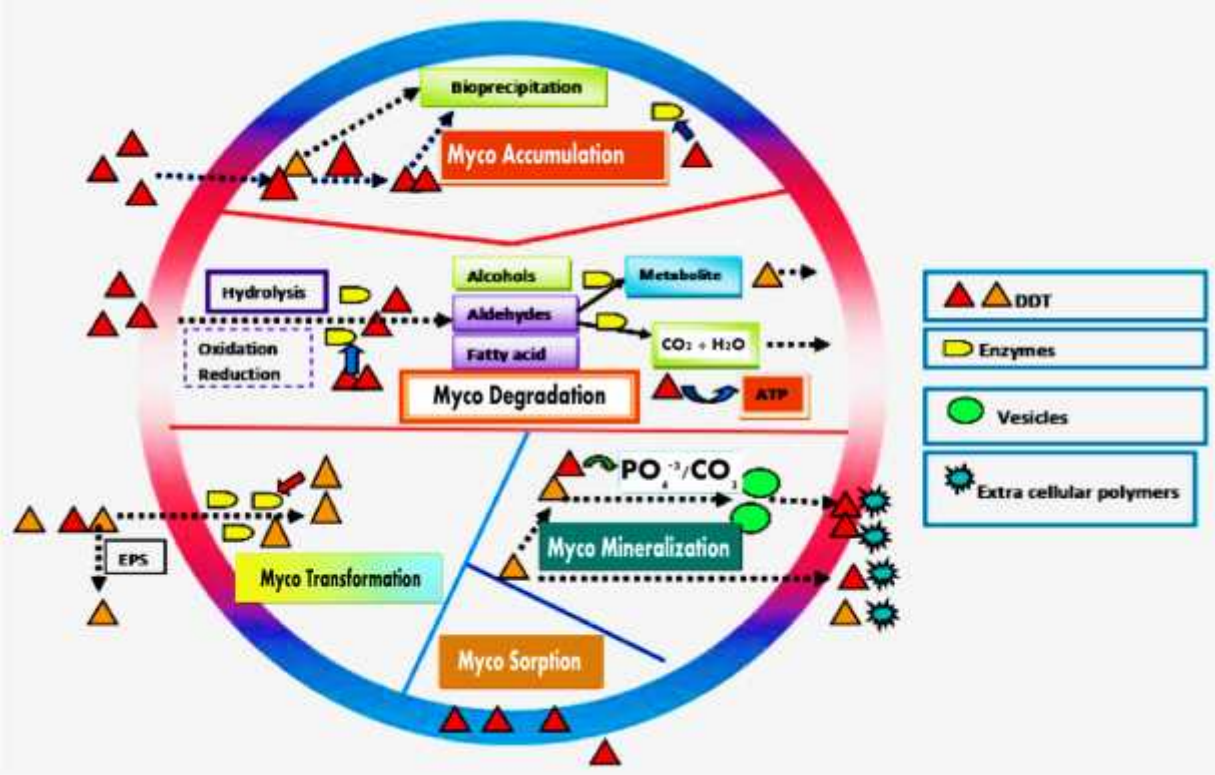


Fig. 1. The mechanisms of fungal remediation used for degradation of DDT pesticides modified from (Ebsa *et al*., 2024).

2.6.1. Fungal DDT Sorption

DDT contaminants bind to fungi by a mechanism known as biosorption, which is independent of metabolism (Shahid *et al*., 2023). Some of these processes involved in fungal sorption include complexation, electrostatic interaction, ion exchange, physical and chemical adsorption, surface adsorption, diffusion, chelation, and microprecipitation (Ahmad *et al*., 2010). Most of this process takes place in the cell wall (Chan *et al*., 2022). The initial barrier preventing DDT contaminants from entering cells is the fungal cell wall, where they may be deposited on the surface or within the wall structure (Yadav and Chandra, 2020; Kumar and Shukla, 2023). The mechanisms of fungal sorption allow hydrophobic substances to cross membranes and enter the organic matrix because of their lipophilic characteristics (Dardiotis *et al*., 2020). Through diffusion and chemical processes such as ion exchange, complexation, and others, contaminants like DDT and its residues

can become adsorbed on cells. Additionally, functional groups (such as amine and hydroxyl groups) contribute to the fungal sorption of DDT contaminants (Chaudhari *et al*., 2023).

2.6.2. Fungal DDT Accumulation

Fungal DDT accumulation is the process by which DDT-pollutants pass through the cell membrane, enter the cytoplasm, and then go through the metabolic cycle of the cell (Shi *et al*., 2023). The combination of physical, chemical, and biological mechanisms is known as accumulation. DDT-fungal accumulation happens when the concentration in the biosphere is significantly higher than in the surrounding area. As a result of fungal accumulation, environmental DDT pollutants harm the fungi (Gizaw *et al*., 2023; Nagar *et al*., 2023). The lipophilic nature of DDT pesticides makes them more easily absorbed in cells due to the lipophilic components on the cell membrane (Behera and Das, 2023). Particulate, insoluble, and by-product DDT pollutants build up in the cellular components of biological cells [Behera and Das, 2023; Nagar *et al*., 2023].

2.6.3. Fungal DDT Transformation

Fungal DDT transformation is the conversion of DDT into another form in the body of fungi by enzymatic reactions (Kamalesh *et al*., 2023). Due to their changed physical and chemical properties, successful mycoremediation of DDT may lead to either a transformation into a low-water-soluble state or a water-soluble and low-hazardous state (Kamalesh *et al*., 2023). The enzymes or metabolites produced by fungi are mostly used in this process. For instance, fungus can create biosurfactants that increase the bioavailability of DDT and enhance the effectiveness of mycoremediation (Behera and Das, 2023; Sheikh *et al*., 2023). In numerous instances, fungi *spp*ecies have shown the efficacy of DDT transformation (Velasco *et al*., 2017; Sheikh *et al*., 2023).

2.6.4. Fungal DDT Degradation

The fungal degradation of DDT involves a key mechanism called metabolism, in which fungi growing at the expense of a growth substrate are able to convert DDT without getting any nutrients or energy for growth (Doukani *et al*., 2022). Fungal degradation of DDT has been documented in different literatures. The majority of reports show that, in reducing conditions, DDT is reductively

dechlorinated to DDD. It has been shown that fungi are capable of metabolizing DDT in this way, and the biodegradation pathways utilizing this method have been established. The strains of fungi that degrade DDT have been identified as an alternate pathway for fungal attack in aerobic environments (Nadeau *et al*., 1994; Gohil *et al*., 2020; Sehrawat *et al*., 2021; and Suman and Tanuja, 2021).

2.6.5. Fungal DDT Mineralization

Fungal DDT mineralization is the process by which DDT and its metabolites are converted into water and carbon dioxide in cells and some tissues under the influence or direction of biological organic matter as shown in Fig. 2 (Bumpus and Aust, 1987). Biologically induced mineralization and biologically regulated mineralization are two mechanisms of fungal DDT-mineralization.

2.7. Fungal Enzymes in DDT remediation

The DDT pesticide-degrading gene resides in an anti-catabolic plasmid and encodes the DDT degradation enzymes (Khaliq, 2023). For instance, in many soil fungi that degrade DDT, the Lin gene encodes many enzymes, including hydrolase, dehydrogenase, and dehalogenase. Fungal genes such as atz, ndo, psb, puh, tfd, tri, and trz encode different groups of enzymes involved in the degradation of DDT. It involves oxidoreductases (such as laccases, oxygenases, and peroxidases) and hydrolytic enzymes (Tarfeen *et al*., 2022). Fungi also release certain catalytic enzymes (including oxidoreductases, oxygenases, monooxygenases, dioxygenases, laccases, and peroxidases) to degrade complex pollutants such as DDT (Tarfeen *et al*., 2022).These enzymes play a role in the DDT mycoremediation process by breaking the chemical bonds and reducing the toxicity of the DDT pollutant (Fig. 3) (Khaliq, 2023).

2.7.1. Laccases Enzymes

Since laccase enzymes are members of the multicopper oxidase family, they are also known as benzenediol dioxygen oxidoreductases or p-diphenol oxidases. They oxidize a variety of substrates while also reducing molecular oxygen to water during the fungal DDT mineralization process (Zainith *et al*., 2020). Insects, higher plants, bacteria, fungi, and other microbes all have laccase enzymes in different quantities for DDT biodegradation mechanisms (Paul *et al*., 2024). In higher plants and fungi, laccases are typically found. However, it has recently been shown that some

bacterial *spp*ecies, including *S. lavendulae*, *Marinomonas mediterranea*, *Pseudomonas spp*., and *Bacillus spp*., also secrete laccases (Guauque-Torres *et al*., 2019; Pandey *et al*., 2023).

The basidiomycetes that produce laccase enzymes are *Phanerochaete chrysospporium*, *Theiophora terrestris, Lenzites betulina, Phlebia radiata, Pleurotus ostreatus,* and *Trametes versicolor* (Pandey *et al*., 2023). These fungi's laccase enzymes break down the complex polyphenolic lignin of DDT and its residue. DDT could be converted into a harmless or nontoxic byproduct using ligninolytic enzymes that can be produced by fungi (Zainith *et al*., 2020).

Furthermore, bacterial laccases can be more active and stable under high-temperature, high-pH, and high-alkaline conditions than fungal laccases (Madbouly *et al*., 2023; Li *et al*., 2024). These enzymes have been gaining attention for biotechnological applications in recent years due to their extracellular and inducible nature, lack of cofactor requirements, and low specificity (Robert *et al*., 2022). The addition of numerous chemicals, including copper, dyes, and other substances, can increase laccase synthesis (Shekher *et al*., 2011). These enzymes allow the breakdown of DDT and its metabolites because of their low substrate specificity. Unlike peroxidases, which use hydrogen peroxide as an electron acceptor, a laccase-catalyzed process utilizes oxygen from the atmosphere (Robert *et al*., 2022). The white-rot fungus laccase, which Polyporus produces, was used to remove DDT from the environment (Gałązka *et al*., 2023). Their findings demonstrated that laccase can efficiently break down DDT after only 25 days of incubation (Gałązka *et al*., 2023).

2.7.2. Oxidoreductases

Fungi use a biochemical mechanism known as the oxidoreductase biocatalyst to absorb energy from the body. This reaction breaks chemical bonds to help transport electrons from the reduced organic component to an alternative chemical molecule (Ekeoma *et al*., 2023; Kumari and Das, 2023). Most fungal *spp*ecies eradicate chlorinated aromatic organic compounds, such as DDT, from polluted areas. It is primarily because of the presence of extracellular oxidase enzymes. These enzymes are found in the adjacent environment and are separated from the fungal body (mycelium). Examples of these enzymes are manganese, lignin, laccase, and peroxidase. Furthermore, as compared to bacteria, fungi could efficiently colonize the soil pollution because of their filamentous structure (Kumari and Das, 2023).

2.7.3. Oxygenases

Oxygenase enzymes belong to the oxidoreductase group. Based on the number of oxygen atoms, oxygenases are classified into two groups: monooxygenases and dioxygenases (Cárdenas-Moreno *et al*., 2023). They are very important in the metabolic process of organic compounds and enhance water solubility (Mahesh *et al*., 2023). These enzymes have a wide range of substrates and are effective against a wide range of compounds, such as DDT compounds (Khaliq, 2023). Oxygenase enzymes break the aromatic rings through the overview of oxygen atoms into organic molecules. It is a very important enzyme in the DDT remediation process (Khalid and Elsherif, 2022). A particular oxygenase enzyme aids in the breakdown of DDT contaminants. Oxygenases also dehalogenate halogenated ethylenes, ethanes, and methanes (Kumari and Das, 2023).

2.7.4. Monooxygenases

Monooxygenases are stereoselective and region-selective enzymes that perform DDT fungal remediation processes as biocatalysts (Karigar and Rao, 2011). Most of the monooxygenase enzymes consist of cofactors, but some monooxygenases do not need cofactors for their function. These enzymes simply need oxygen for their achievements (Ebrecht *et al*., 2023). Monooxygenases participate in the decomposition of hydrocarbons, viz., alkanes, cycloalkanes, methanes, alkenes, haloalkenes, aromatics, and ethers (Singh *et al*., 2021). Monooxygenases carry out the oxidation of dehalogenation reactions and dehydrochlorination in oxygen-rich environments. However, in oxygen-less environments, the reduction of dechlorination reactions is carried out. The oxidation of the substrate during dehalogenation results in labile products, which are then chemically decomposed (Qin *et al*., 2023).

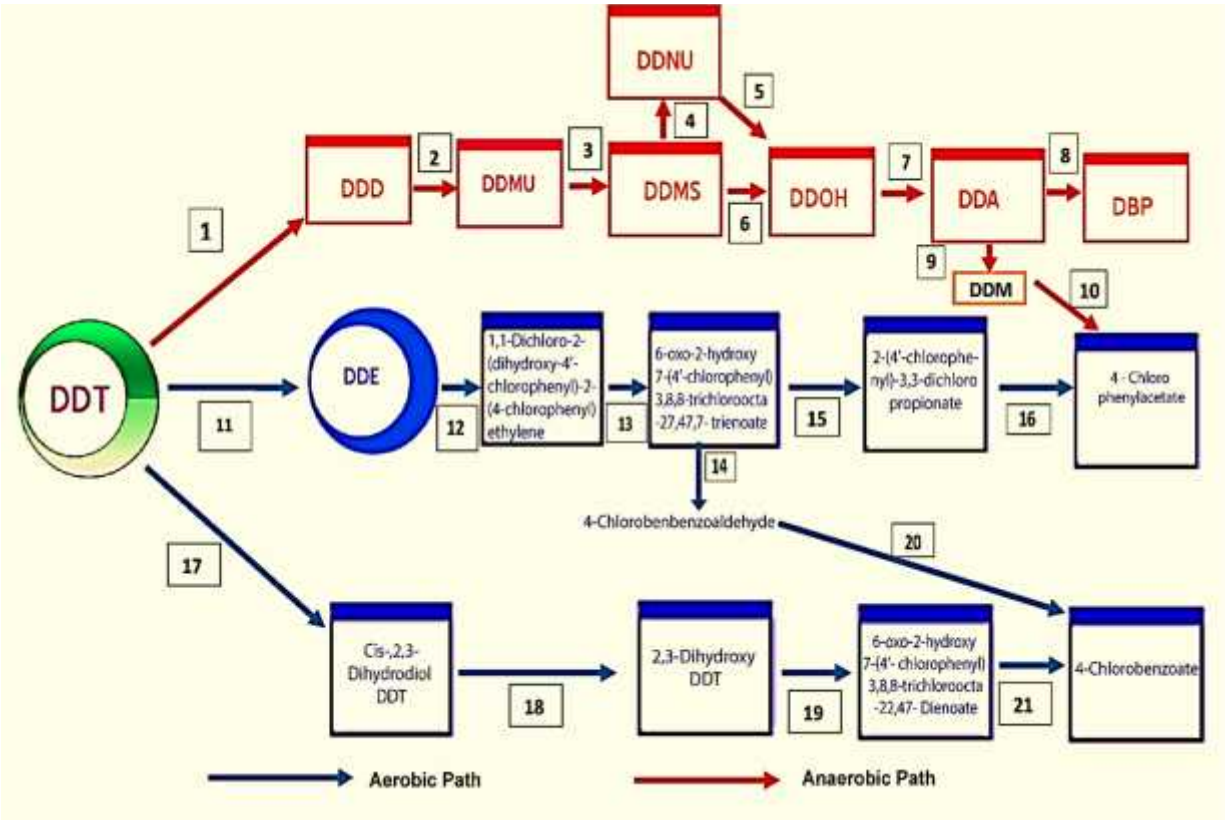


Fig. 3. Mechanistic scheme of DDT enzymatic microbial degradation under anaerobic condition (red) path way DDT reductive dechlorinase (1);DDD dehydrochlorinase (2); DDMS dehydrogenase (3); DDNU hydratase (5); DDMS dehalogenase (6); DDA decarboxylase (8); DDA decarboxylase (9); DDE dehalogenase (22) and under aerobic conditions (blue) pathway DDT dehydrochlorinase (11), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene dioxygenase (13, 14), 6-oxo-2-hydroxy 7-(4'-chlorophenyl) 3,8,8-trichloroocta-2Z,4Z,7-trienoate hydrolase (15), 4-chlorobenzaldehyde dehydrogenase (14), 4-chlorobenzoaldehyde dehydrogenase (20), DDT 2,3-dioxygenase (17), cis-2,3-dihydrodiol DDT dehydrogenase (18), and 2,3-dihydroxy DDT 1,2-dioxygenase (19) modified from (Ebsa *et al*., 2024).

2.8. Fungal DDT Remediation Strategies

DDT remediation strategies for detoxification of DDT mainly depend on physical, chemical, and biological approaches. These approaches can be used alone or in combinations (Bakshi *et al*., 2021; Parial and Dey, 2023). Cleaning DDT from the environment can be carried out adequately, both *Ex situ* and *in situ*, according to the need or not to remove contaminated soil (Hussain *et al*., 2022).

2.8.1. *In Situ* Fungal DDT Remediation Strategies

In situ fungal remediation techniques are preferable over *Ex situ* techniques for the treatment of DDT-contaminated water and soil ecosystems due to their low cost**,** minimal maintenance, environmental friendliness, and sustainability (Hussain *et al*., 2022). *In situ* fungal DDT remediation can be done in three different ways: myco-attenuation, myco-stimulation, and myco-augmentation (Bose *et al*., 2021).

2.8.1.1. Myco-attenuation Approach

DDT toxic contaminants are converted into less toxic or non-toxic forms by fungal degradation, with their interaction with naturally existing chemicals and their sorption on geologic media, which can facilitate bio-attenuation. This mechanism is efficient and cost-effective, but it also takes a long time, depending on the conditions of the DDT-contaminated location. It may not require a complete cleaning up of DDT (Raffa and Chiampo, 2021; Chaturvedi *et al*., 2023).

2.8.1.2. Myco-stimulation Approach

The method of changing the environment to promote the growth of fungi capable of DDT remediation is known as myco-stimulation. This can be achieved by supplementing insufficient amounts of electron acceptors and limiting nutrients such as carbon, nitrogen, phosphorus, or oxygen, which restrict fungal activity. The detoxification potential for DDT pesticides under liquid media conditions has numerous factors that affect fungal growth. These factors are nutrients, pH, temperature, moisture, oxygen, soil characteristics, and the presence of DDT pollutants, which might prevent fungal DDT degradation in soil and water.

The primary advantage of myco-stimulation is that fungal remediation of DDT will be carried out by indigenous fungi that have already existed, are widely dispersed, and are well-suited to the subterranean environment (Gonçalves *et al*., 2022). Both mycoaugmentation and fungal-remediation techniques can work together to enhance the ability of fungi to degrade DDT compounds and optimize the efficiency of the process (Shahbaz *et al*., 2023).

2.8.1.3. Mycoaugmentation Approach

Myco*a*ugmentation is a green technology that is known as an enhancement of fungi DDT degradation capability by applying it to specific contaminated areas (Wang *et al*., 2022). Specific kinds of fungal groups can enhance DDT breakdown significantly. This technology is mostly applied in areas where inhabitant microorganisms do not have a significant degradation capability of DDT (Jyoti *et al*., 2023). Mycoaugmentation is mainly enhancing the catabolic potential of the fungi for the recovery of contaminating agents (DDT). To accomplish this objective, the inoculation of desired fungal *spp*ecies can be carried out. Besides these, genetically modified and engineered organisms (GMOs) are extremely suitable for DDT degradation and enhanced mycoaugmentation processing.

Several fungal growth variables affect mycoaugmentation. An appropriate strain selection is a very important factor. Therefore, planning inoculum development should also be taken into consideration (Liu *et al*., 2021).The strains should have a high potential for DDT degradation, and the selected strain could colonize the DDT-contaminated area within a short period of time (Zulfiqar *et al*., 2023).The development of strains has a higher capacity to survive under high DDT concentrations (Wang *et al*., 2023; Wu *et al*., 2023;Ebsa *et al*., 2024).Furthermore, the strain can efficiently survive under a wide range of harsh and suitable environmental conditions (Wang *et al*., 2023).

New fungal *spp*ecies should be investigated that have better and faster degradation capacity in contaminated environments (Bhatt *et al*., 2021).Isolating fungi from DDT-contaminated ecosystems is a good source for *myco*augmentation (Muter *et al*., 2023). It is crucial to determine how DDT pesticide detoxifying capacity in liquid media settings contributes more effectively to the mycoaugmentation of fungal consortiums (Gomathi *et al*., 2023).

2.8.2. *Ex-situ* fungal DDT Remediation Strategies

Ex-situ fungal DDT remediation of water and soil demands the laborious, expensive, and time-consuming removal of DDT-contaminated water and soil from the site to be treated elsewhere (Kuppusamy *et al*., 2016; Hussain *et al*., 2022). Bioreactors can also be categorized as an *ex-situ* fungal remediation technique (Kuppusamy *et al*., 2016). The use of bioreactors in fungal

remediation techniques provides a number of advantages. These are better control and management of factors like temperature, pH, agitation, and aeration, as well as the option of enhancing the process by incorporating different optimization strategies (Bala *et al*., 2022).

Fungi utilize DDT as a carbon source, which mostly depends on the presence of DDT residues and fungi in the soil (Zhang *et al*., 2020). The retention of DDT residues with colloidal particles can significantly make them unavailable to fungi, which will affect their degradation. More than 10,000 fungal colonies are present in 1 g of bulk soil, approximately. Some of which are highly involved in DDT residue degradation in the ecosystem over time (Yu *et al*., 2023). Fungal *spp*ecies used in microbial DDT remediation techniques are obtained from bulk soil and decaying wood. Wang *et al*. (2022) investigated thirteen fungal *spp*ecies in the removal process of DDT residues, which included Ascomycota (7 *spp*ecies), Basidiomycota (4 *spp*ecies), and Glomeromycota (2 *spp*ecies) (Wang *et al*., 2022). Some potential fungal species have been identified as involved in the breakdown and detoxification of DDT as shown in Fig. 4.

Fig. 4, Fungal remediation of DDT from waste water and farm soil modified from (Ebsa *et al*., 2024).

2.9. Trends in Enzymatic DDT Degradation Methods

2.9.1. Enzyme Immobilization

Fungal enzymes that have been immobilized are used in a wide range of scientific and environmental cleaning procedures to remove persistent organic pollutants like DDT (Khaliq, 2023). Enzymes are immobilized into strong, stable supports to maintain high stability in terms of pH, temperature, packaging, reuse, and separation (de Melo *et al*., 2023). A Saravanan *et al*. (2021) reported that horseradish peroxidase (HRP) was cross-linked onto calcium-alginate beads using glutaraldehyde as a cross-linking agent to help with the covalent immobilization of HRP, which is necessary for the breakdown of DDT.

2.9.2. Genetic Engineering Approach

Enzyme utilization is restricted in industrial-scale environmental applications due to the economics of enzyme processing (Bello *et al*., 2021). The researchers used genetic engineering methods to increase the yield of enzyme production to remediate environmental pollutants such as DDT (Rafeeq *et al*., 2023). This can be achieved using one of two methods: genetic experimentation to pick natural forms or mutagenesis of a gene *in vitro* and expression in a host cell. Laccase enzymes are predominantly employed for genetic manipulation. The laccase gene from *A. niger* cDNA was used for cloning for DDT degradation purposes (Gao *et al*., 2010).

2.10. Main Factors Affecting Fungal DDT Remediation

In order to sustain the high bioavailability of fungi in soil and water for the complete DDT remediation capability of fungi, a specific combination of biotic and abiotic variables is required (Chaudhari *et al*., 2023). A number of variables affect the complete fungal DDT remediation process. The same variables that have an impact on the fungal remediation of contaminated soils and waters also have an effect on the mycoremediation of DDT-contaminated soil and waters. Abiotic and biotic variables are most likely to have an impact on the fungal remediation of DDT-contaminated soil and water (Al-Rashed *et al*., 2021).

2.10.1. Abiotic Factors

The fungal remediation of DDT could be significantly affected by abiotic factors such as pH, temperature, soil type, water, soil moisture, cation exchange capacity (CEC), redox potential (Eh), nutrient sources, exudate quantity, rhizosphere environment, and the composition and biochemical processes. These factors can influence the fungal growth in DDT-contaminated soil and water environments (Zhang *et al*., 2020; Al-Rashed *et al*., 2021). DDT becomes more soluble at higher temperatures, which increases the pesticide's bioavailability (Zhang *et al*., 2020; Kibria *et al*., 2021; and Rafeeq *et al*., 2023). Furthermore, insufficient soil moisture can make the fungal DDT remediation process more difficult. Low soil moisture content hinders the growth and metabolism of fungi, and high values reduce soil aeration (Chaudhari *et al*., 2023).

The type of soil has a significant impact on the bioavailability of DDT remediation in the soil (Baxter and Cummings, 2008). The texture of soil particles has a direct impact on the availability of potential DDT-degrading fungi (Rashmi *et al*., 2022). Loam and sand have the maximum DDT availability, while fine-textured clay soils and clay loam have the lowest availability (Pradhan *et al*., 2023). Clay content, oxygen concentration, salinity, minerals, and the availability of nutrients can all have an impact on fungal DDT remediation (Chugh *et al*., 2022). Organic matter content and soil types can influence how fungi react to DDT pollutants (Krithiga *et al*., 2022).

The enzymatic dosage has played a crucial role in the fungal DDT remediation process (Kamalesh *et al*., 2023). Typically, DDT degradation efficiency rises to a certain point with elevated enzyme concentrations (Saravanan *et al*., 2021). Consequently, DDT remains persistent due to the limited concentration of fungal enzymes available both in the batch and environmental system (Saravanan *et al*., 2021). Agitation is also considered a crucial factor for DDT-microbial remediation, as it is primarily responsible for heat, substrate, and oxygen transfer in the process. A higher speed of agitation results in enhanced biodegradation efficiency owing to better oxygen availability in aerobic processes. Speeds of 200 rpm/min resulted in increased DDT-degradation potential (Saravanan *et al*., 2021).

2.10.1.1. pH

pH has an effect on fungal DDT remediation because different fungal *spp*ecies have different optimal pH ranges. Soil pH can have a variety of effects on fungal DDT remediation (Shahid *et al*., 2023). At extreme pH, some fungal degradation processes are inhibited. Some still have the potential to remedy DDT pollution in suboptimal conditions (Alvarez *et al*., 2021). The optimal pH varies for different enzymes derived from different microorganisms. A pH value outside the optimal range results in lower enzyme activity. Most of the enzymes work in a neutral pH range. However, some enzymes, such as alkaline proteases, work at pH levels above 8.0. It was studied how lignocellulolytic enzymes from white-rot fungi, namely manganese peroxidases (MnP) and lignin peroxidases, broke down DDT through fungal degradation. For crude enzymes, the optimum pH for fungal degradation has been found at pH 5 (Salem *et al*., 2023).

2.10.1.2. Temperature

Temperature has an impact on the chemical composition as well as the physical properties of DDT contaminants (Jones, 2021). Temperature has a direct impact on how DDT pesticide adheres to fungi, soil, and particles during the adsorption and desorption processes (Dewangan *et al*., 2021). With rising temperatures, DDT's adsorption capacity and intensity will increase (Amutova *et al*., 2023). The DDT breakdown process by fungi benefits from an increase in temperature within a suitable range (Pande *et al*., 2020). Temperature affects the activity of both intracellular and extracellular enzymes of fungi in DDT degradation mechanisms (Wang *et al*., 2023). Fungal enzymes have a variety of optimal enzymes for DDT degradation (Ghosh and Sarkar, 2023). Because enzymes are proteins, they are especially susceptible to high temperatures, which may cause the protein to become denatured or alter its characteristics (Wang *et al*., 2023). It was discovered that *Asppergillus spp*. that breaks down DDT thrives best in temperatures between 25 and 35 °C. This is because higher temperatures cause the laccase enzymes to either become inactive or cleave (Wang *et al*., 2010). The immobilized enzymes produced extracellularly by white-rot fungi demonstrated remarkable activity and stability across a wide temperature range (20 to 55°C). This biocatalyst effectively degraded p,p'-DDT, completely eliminating it within a 12-hour incubation period at 30°C (Salem *et al*., 2023).

2.10.1.3. DDT Effects on Mycoremediation

DDT pesticides can directly affect fungi as well as mycoremediation procedures (Kamalesh *et al*., 2023). Some fungi can thrive in DDT-contaminated areas, despite the potential toxicity, due to resistance or tolerance mechanisms (Anju *et al*., 2010; Chugh *et al*., 2022; Ebsa *et al*., 2024). However, it has still variable degrees of effect on its biomass production, physiological morphology, growth, fungal activity, and mycelia colonization (Chugh *et al*., 2022). DDT pesticide exposure may change the physiological and biochemical behavior of soil and water microorganisms (Chugh *et al*., 2022; Kumar *et al*., 2023). DDT insecticides interfere with some crucial processes and inhibit a number of enzymes, disrupting the mechanism that fungi use to promote their growth (Gupta *et al*., 2022; Kumar *et al*., 2023).

DDT has an impact on the number of fungal *spp*ecies, biomass production, and shape of fungi (Gupta *et al*., 2022; Ebsa *et al*., 2024). Notably, while DDT is hazardous to some fungal *spp*ecies, some fungi can utilize DDT pesticides in low concentrations as a source of nutrients or energy to reproduce and promote development throughout the mycoremediation process (Dad *et al*., 2022; Paul *et al*., 2024; Ebsa *et al*., 2024). Occasionally, over time, fungal populations can develop tolerance or resistance to DDT, respond to normal conditions, or even increase DDT concentration. (Paul and Mandal, 2019; Mertens, 2006; Ebsa *et al*., 2024).

2.10.2. Biotic Factors

The ability of DDT mycoremediation in soil and water is determined by biotic factors such as inoculum density, survival, colonization, competitiveness, fungal activity, physical diffusion capacities, etc. (Mertens, 2006; Melendez-Pastor *et al*., 2023). The success of DDT bioremediation depends on the *spp*ecies' ability to survive in these stressful environments. A major factor limiting the effectiveness of fungal DDT remediation and strongly influencing the success or failure of mycoremediation is bioavailability. These factors influenced biological activity like *spp*ecies types, fungal biomass, symbiotic *spp*ecies, and bioavailability (Al-Rashed *et al*., 2021; Shahid *et al*., 2023).

3. MATERIALS AND METHODS

3.1. Study Area Description

The samples of soil, effluent, and wastewater were taken from East Shewa, mainly Ziway, Koka, and Addis Ababa. The study site was chosen because of its history of DDT pesticide dumping, significant agro-industrial waste disposal sites, irrigation from polluted rivers, and significant urban agricultural farm activities. With geographic coordinates of 8^0 9′ N, 38^0 49′ E latitude, 38◦ 42′ 59.99″ E longitude, and an elevation of 1645.250 m above sea level, Ziway City is situated in the Ethiopian Rift Valley. At an elevation of 1590 meters above sea level, Lake Koka is situated in the Ethiopian Rift Valley (08°23'22" N; 39°05'15" E), about 90 kilometers southeast of Addis Ababa. Addis Ababa is situated at 9°1′48″N and 38°44′24″E, 2,355 meters (7,726 feet) above sea level, as shown in Fig. 5.

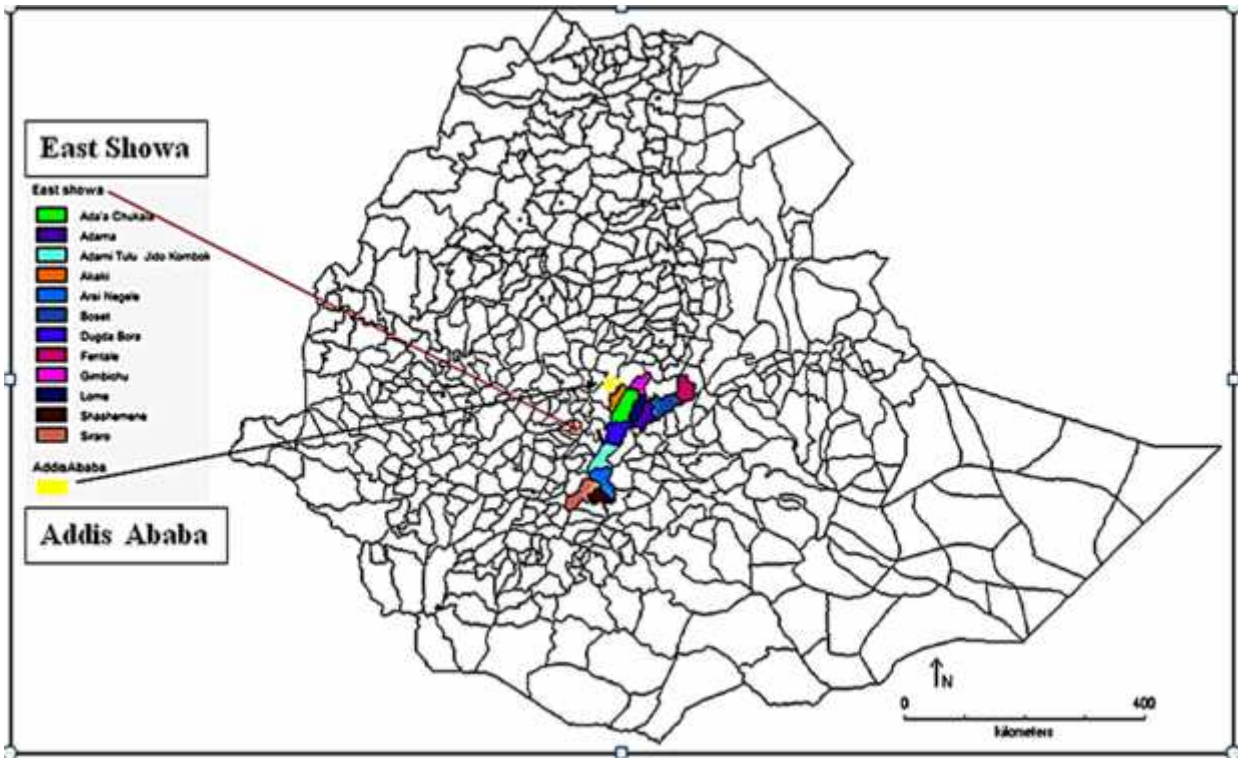


Fig. 5. Map of Study area

3.2. Chemical Standards

DDT (98%), DDD (99%), and DDE (99%) standards having such purity for GC-ECD analysis grade were purchased. from (Taufkirchen Sigma-Aldrich Chemie Germany) For GC-ECD (gas chromatography electron capture detector) detection standards, used for DDTr analysis, were prepared by diluting each of the individual DDT, DDD, and DDE standards, and the DDT residues (DDTr = DDT, DDD, and DDE) were utilized. Analytical laboratory DDT was acquired from the pesticide processing factory in Ethiopia. The chemicals and solvents used in the experiment were all of analytical grade. The major equipment that was used for the GC-ECD analysis sample preparation included analytical balances (±0.01 mg) (Sartorious AG, Germany), a refrigerator centrifuge (HERMLE, Z446K; Germany), a nitrogen concentrator (Multi Vap 54 Lab Tech, USA), a wrist action mechanical shaker (model 75, Burrell Scientific, LLC, Pittsburgh, PA, USA), a vortex mixer (Biocote Sturt, Italy), micropipettes, Duran bottles, bottle top dispensers, beakers, various class A volumetric flasks and measuring cylinders, and a pH meter (Bibby Scientific Ltd, U.K.). All glassware and other apparatuses were scrupulously cleaned and dried in a drying cabinet (LEEC, Limited, UK).

3.3. Experimental design

This study consists of three experiments: a screening assay, an optimization assay, and a degradation assay. There were two factors involved in the DDT pesticide resistance assay. The amount of DDT concentration ranged from 100 ppm to 10,000 ppm, and the media type (DDT-amended, non-amended, or control media) with 40 DDT-tolerant fungal *spp*ecies. The optimization assay were carried out using a four-factor design with three levels. This comprises two DDT concentrations (3500 ppm and 7000 ppm) and a control (no DDT), along with fungus *spp*ecies (AS1, AS1T1, and T1), temperature (25, 30, and 35℃), and pH (4, 7, and 10). In this study, response surface designs based on Box-Behnken produced 45 experimental runs, including one replication experiment. To assess the DDT degradation studies, a one-way ANOVA with a two-factor design was utilized. One replication experiment and a total of 27 experimental runs were generated by the ANOVA analysis. The two assay involved in this experiment were media type (1750 ppm DDT, 3500 ppm DDT, 5250 ppm DDT, and 7000 ppm of 100% pure DDT broth media supplemented with minimal salt medium), (1:1 w/w PDB + 1750 ppm DDT, PDB + 3500

ppm DDT, PDB + 5250 ppm DDT, and PDB + 7000 ppm DDT) and the second assay were DDT and its metabolite (DDE and DDD) analysis.

3.4. Sample Collection

Using the stratified random sampling method, a composite of 100 soil samples from Addis Ababa (40), Koka (30), and Ziway (30) with the help of a sterilized spatula were collected from agro-industrial waste by plucking up to 5 cm of topsoil. Similarly, 50 effluent samples from Addis Ababa (20), Koka (15), and Ziway (15) were collected. The samples that were obtained were stored in a refrigerator in a sterile 45-mL Falcon tube. All sample tubes were taken to the mycology laboratory at Addis Ababa University and then kept in the refrigerator at +4 until processing, which of each contained 25 mL of effluent wastewater and 25 grams of soil.

3.5. Screening of DDT Tolerant Fungal Isolates

The minimal salt medium (MSM) used in the DDT degradation experiment had the following g/L amounts of salt: 1.65 g NaCl, 0.19 g K_2HPO_4, 0.188 g $CaCl_2$, 0.198 g KH_2PO, and 0.396 g $NH_4(SO_4)_2$ modified from the method of Nadeau *et al*. (1994) were added to five 500-mL Erlenmeyer flasks, each containing 225 mL of distilled water (pH 7.0) contained 25 g of pooled soil or effluent sample and supplemented with 100 ppm DDT, then incubated at 25±2℃ in an incubator shaker (New Brunswick Innova® 42) at 120 rpm for 15 days. The concentration of DDT was increased stepwise to expose fungi to high concentrations ranging from 500 ppm up to 10,000 ppm.

The dilution plate method was used to isolate fungi from these DDT-enriched 15-day-old samples (soil or effluent water ratio of 1:1000). From each, suspension 0.1 mL portions were transferred into potato dextrose agar growth media (PDA; Hi-Media, Mumbai, India) (Persiani *et al*., 2008). The isolates were purified and identified using traditional taxonomic keys based on macro- and microscopic features after 5 days of incubation at 25 ± 2°C on PDA (Bedine *et al*. and Susila *et al*., 2023). The pure isolates were cryopreserved at +4°C for further analysis with 50% glycerol and 50% PDB at the mycology lab of Addis Ababa University.

The tolerance screening was performed in solid media containing various concentrations of DDT and media types. It includes 100 ppm, 500 ppm, 1,000 ppm, 1,500 ppm, 3,000 ppm, 5,000 ppm, 7,000 ppm, and 10,000 ppm of 1:1 PDA and DDT, as well as 100% pure DDT supplemented with a mineral salt agar medium. Below 0.1 mL, acetone was added to the final test medium as per the Organization for Economic Cooperation and Development's recommendation (Rasmussen *et al*., 2019). The initial minimum DDT concentration (100 ppm) was determined depending on recent published papers (Al-Rashed, 2021), and the maximum DDT concentration (10,000 ppm) was tested to determine the fungal maximum tolerable limit or resistance capacity. Following careful evaluation of tolerance screening, final DDT concentrations of 0 ppm, 3500 ppm, and 7000 ppm were determined for further optimization studies. Tests without DDT were used as a control in the experiment. The optimization was performed in 250-mL Erlenmeyer flasks of broth media.

DDT-tolerant fungal isolates were chosen based on fungal growth characteristics, biomass output, and sporulation capacity (Domsch *et al*., 2007). To determine fungal tolerance to DDTs, two indices were used. The tolerance index (TI) is calculated based on the dry weights (DW) of fungal mycelia biomass production to evaluate fungal growth in the presence or absence of DDTs, as indicated by equation (1). To assess fungal reproduction in the presence or absence of DDTs, another index called the sporulation index was used based on the ability of the fungi to sporulate (Ceci *et al*., 2015).

$$\%\text{Tolerance index (TI)} = \frac{\text{DW of treated mycelium}}{\text{DW of control mycelium}} \times 100 \quad \ldots\ldots (1)$$

3.6. Fungal Identification

3.6.1. Macroscopic and Microscopic Examination of Fungal Isolates

The final chosen fungal isolates were identified based on morphologic characteristics (macro and microscopic features) and bimolecular characterization by using MALDI-TOF mass spectrometry analysis with the Bruker MALDI Biotyper microflex LT (Bruker Daltonics, Bremen, Germany) (Silva *et al*., 2023). The chosen fungal isolate was subcultured onto potato-dextrose agar (PDA; Hi-Media, Mumbai, India) and inoculated in the middle of each plate using a 5 mm-diameter cork borer agar block. The plates were then incubated at 25±2℃. Until the plates were completely covered in fungal growth, the radial extension of the fungal mycelia was measured every 24 hours

with a ruler; the color, reverse side, growth condition, margins, and elevation were noted, and macroscopic images were captured using a Nikon D 5200 camera. Macroscopic identification was done using the morphologic key of a pictorial atlas of soil and seed fungi (Watanabe, 2010). Microscopic observations were made from plate and slide cultures on PDA incubated for five days. Spore shape, hyphal nature, conidia, and conidiophores were investigated. Slides were mounted using lactophenol cotton blue (LPCB) and examined using an Olympus CETI light microscope (Olympus Keyence International, Bedrijvenlaan, Belgium) (Silva *et al*., 2023). Microscopic images were captured using a full-frame sensor camera (Canon 5Ds Mark III Thailand).

3.6.2. MALDI-TOF Mass Spectrometry Analysis

Fungal identification using MALDI-TOF MS is based on the ionization of fungal proteins by a laser, followed by the creation of a spectrum representing a *spp*ecies-specific protein fingerprint by comparing their spectra with Andromas or the Vitek MS database reference libraries (Vermeulen *et al*., 2012). The fungal isolates were cultured on potato dextrose agar (Becton Dickinson GmbH, Heidelberg, Germany) and incubated at 25±2℃ for three days until sufficient growth was observed. Bruker MALDI Biotyper microflex LT (Bruker Daltonics, Bremen, Germany) was used for fungal identification (Huang *et al*., 2023).

3.6.2.1. Extraction and Sample Preparation Procedure

The front mycelium was placed into 1 mL of HPLC water, centrifuged to pellet it, and then the pellet was resuspended in 300 µL of HPLC water in an Eppendorf tube. One µL of inoculation loop mycelia were isolated, transferred from the culture plate into the water, and mixed thoroughly until the material was completely suspended. The microbial material on a benchtop was then centrifuged for 2 minutes at 13,000–15,000 rpm after 900 µL of ethanol was added and properly mixed. The supernatant was removed using a pipette, avoiding contact with the microbial materials. The pellet was air-dried for five minutes at room temperature. Twenty-five µL of 70% aqueous formic acid was added, and the pipette solution was moved up and down until the pellet was suspended. The tube was centrifuged for 2 minutes at 13,000–15,000 rpm. A microliter of supernatant was deposited onto a vacant sample position of a MALDI target plate, dried at room

temperature, overlaid with 1 µL of HCCA matrix, and analyzed with MALDI-TOF MS (Hleba *et al*., 2022; Tsuchida *et al*., 2023).

3.7. Compatibility Test among the Selected Fungal Isolates

To study the compatibility test of isolate AS1 with T1-paired mycelia, T1 and AS1 were inoculated 4 cm apart from each other in the same Petri dish. The co-inoculated plates were incubated at 27±2℃ for 3 days. Each fungus was grown separately under the same conditions, and its growth rates were compared. The expansion rate of each fungus was evaluated. The interactions between the two mycelia were assessed according to the method by Molla *et al.* (2001).

3.8. Fungal Biomass Production and Sporulation Determination Assay

Three media types were identified as having strong effects on the fungal biomass production and sporulation and finally chosen for optimization of crucial growth factors. These are 1:1 PDB and DDT, 100% DDT supplemented with minimal salt, and PDB (non-DDT-amended media). The capacity of dry biomass production and sporulation was determined for three different fungal isolates (AS1, T1, and AS1T1) at different temperatures (25, 30, and 35℃), DDT concentrations (0, 3500, and 7000 ppm), and pH values (4, 7, and 10). For both dry biomass production and sporulation response, 45 runs were performed with three levels of representation for each variable. The statistical experimental tool of Box-Behnken design was used for the selection and improvement of crucial growth factors (Govarthanan *et al*., 2022).

3.9. Identification of Critical Growth Factors

Three media types 1:1 PDB and DDT amended, 100% DDT amended, and non-amended media were identified as having strong effects on the production of dry biomass and sporulation and finally chosen for optimization of crucial growth factors based on the results of the secondary screening test. The ability to produce dry biomass and produce spores was determined for three different fungal isolates (AS1, T1, and AS1T1) using constant incubation periods at different temperatures and DDT concentrations (0, 3500, and 7000 ppm), pH values (4, 7, and 10), and temperatures of 25, 30, and 35℃. For both dry biomass production and sporulation response, 45 runs were performed with three levels of representation for each variable. The statistical

experimental tool of Box-Behnken design was used for the selection and improvement of crucial growth factors (Govarthanan *et al*., 2022).

3.10. Statistical Optimization Biomass Production and Sporulation for Identification of Significant Variable Using Response Surface Methodology (RSM)

Response surface methodology (RSM) based on Box-Behnken design (BBD) are experimental statistical tools used to study the impact of abiotic factors on biotic factors for the production of fungal biomass and sporulation (Govarthanan *et al*., 2022). Optimizing growth factors increases the biodegrading capacity of microbes in high concentrations of DDT. This statistical tool can be utilized to optimize both factors (Chen *et al*., 2022; Thirumoorthy *et al*., 2023). RSM has been widely used as an optimization tool, with both having different perspectives on modeling different process parameters (Hamidi *et al*., 2023; Malla *et al*., 2023). Four independent factors with three levels were identified. These were fungal isolates (AS1, AS1T1, and T1), DDT concentration (0, 3500, and 7000 ppm), temperature (25, 30, and 35℃), and pH (4, 7, and 10). Box-Behnken is a class of response surface designs that generates a total of 45 experimental runs, with one replication experiment in this study. The fungal isolates AS1, AS1T1, and T1 were grown in 1:1 PDB+DDT at concentrations of 3500 and 7000 ppm and PDB as a control medium, respectively. For the quantitative information acquired from the 45 experimental runs, BBD employed multiple regression analysis. The optimization study was conducted to assess and choose significant abiotic and biotic variables for the best conditions for the formation of fungal biomass and sporulation. The Minitab 2019 software (Minitab Inc., State College, PA, USA) was used to generate the optimization experiments.

3.11. DDT Degradation by Fungal Co-Cultures *A. niger* and *T. koningii*

Mycelia of *A. niger and T. koningii* were harvested from 10-day-old PDA cultures by washing with sterilized distilled water. The amount of spores was adjusted using a hemocytometer (Neubauer chamber, Taufkirchen, Germany) and an Olympus CETI light microscope (Olympus Keyence International, Bedrijvenlaan, Belgium). *T. koningii* and *A. niger* were co-inoculated in each 500-mL flask of DDT-amended MSM. The inoculum level was 10^8 spores/mL; each medium contained 100 mL of volume culture media, and the culture medium was then incubated at 27 ± 2℃ in an incubator shaker at 120 rpm for 15 days (Wilkes *et al*., 2021).

In order to investigate the DDT breakdown efficiency of fungal co-cultures *A. niger* and *T. koningii*, four different DDT initial concentrations were tested in two media types (1:1 ratio PDB+DDT and 100% pure DDT supplemented with MSM). *A. niger* and *T. koningii* were co-inoculated in a 100-mL volume of DDT-amended MSM at 1750, 3500, 5250, and 7000 ppm DDT initial concentrations. After incubation, a sterile membrane containing filter paper with a 0.22 µm pore size was used to separate the mycelia from their liquid media. The filtrates were then centrifuged at 10,000 rpm for 5 minutes. The supernatant was diluted using sterilized distilled water to 25, 50, 75, 100, and 125 ppb, and 50 mL were taken for GC-ECD analysis.

3.12. GC-ECD Analytical Methods

The degradation of DDT was analyzed using gas chromatography (GC-ECD, Agilent 7890A, USA) coupled with a 63Ni electron capture detector (ECD) and a packed column HP-5-5% phenyl methyl siloxan (30 m x 32 m i.d., film thickness 0.25 µm). The oven procedure was as follows: The initial temperature of the oven was 75, gradually increasing at 25/min to up to 150, held for 0 min, then increasing at 5/min to 280, held for 10 min. The total run time was 39 minutes. Nitrogen (99.995% purity) was used as a carrier gas at a flow rate of 60 mL/min. From an aliquot of 0.5 mL, 2 microliters were injected in splitless mode. Chemstation software was used for instrument control and data analysis (Liu *et al*., 2015).

3.12.1. Analysis of DDT, DDD and DDE

DT residues were analyzed using gas chromatography (GC-ECD) coupled with an electron capture detector (ECD). Its packed column of HP-5-5% phenyl methyl siloxan (30 m x 32 m i.d., film thickness 0.25 µm). The oven conditions used were the same as reported previously (Liu *et al*., 2015). Minitab 19's ANOVA was used to assess the percentage degradation as indicated in equations (2, 3, and 4). The degradation efficiency of DDT and DDTr was calculated using the following equation (Ma *et al*., 2016).

$$\%\ \text{DDT Break down} = \frac{(\text{Peak area DDE} + \text{Peak area DDD})}{(\text{Peak area DDE} + \text{Peak area DDD} + \text{DDT})} \times 100 \quad \ldots\ldots (2)$$

$$\%\ \text{DDE Break down} = \frac{(\text{Peak area DDD} + \text{Peak area DDT})}{(\text{Peak area DDE} + \text{Peak area DDD} + \text{DDT})} \times 100 \quad \ldots\ldots (3)$$

$$\%\ \text{DDD Break down} = \frac{(\text{Peak area DDE} + \text{Peak area DDT})}{(\text{Peak area DDE} + \text{Peak area DDD} + \text{DDT})} \times 100 \quad \ldots\ldots (4)$$

2.13. Statistical Analysis

The design matrix was generated, and the results were analyzed using mathematical modeling. The DDT tolerance optimal conditions were identified using Minitab 2019 software (Minitab Inc., State College, PA, USA). Preliminary experiments were used to establish the upper and lower limits for each independent variable. Response surface methodology (RSM) based on the Box-Behnken design (BBD) was used to calculate the amount of dry biomass of fungal mycelia in grams, and an ANOVA was performed to identify significant variables using Minitab version 19 software. It was carried out to ascertain the impact of chosen parameters on fungal dry biomass production and sporulation. The effect of the media type, temperature, and pH on the response was examined using a Tukey HSD test. The designed model was also validated using probability (p-value) values, and the p-value shown is < 0.05, which is regarded as a significant model term. The designed model's F-value of 2.13 suggests that it is significant. The high F-value could be due to noise. Additionally, the lack of fit score (5.235) for the model that was developed implies that the lack of fit is not significant. The fact that the model's terms don't fit significantly indicates that the model fits well.

The fungal biomass and sporulation were accurately and consistently measured by the batch experiments, and the R (0.572) further confirmed the model's significance. The response surface model fits the experimental data to identify the interaction between the factors and the response (Mani *et al*., 2023).The spore/mL results were log-transformed (base 10). Fisher tests were used to compare all treatment means at $p < 0.05$. The statistical significance has been studied through the use of multiple regression analysis techniques. The F-test was used to assess R^2. The significance of independent variables and their interactions was tested using RSM based on Box-Behnken design analysis. The standardized effects of the independent variables and their interactions with the dependent variables were also investigated using a Pareto chart. Results were assessed with various descriptive and inferential statistical tools, i.e., p-value, F-test, R^2, R^2adj, sum of squares (SS), and mean sum of squares (MSS) tests, to assess the goodness-of-fit of the developed mathematical model to the experimental data (Aziz *et al*., 2014). At a 95% level of confidence, differences between means were regarded as statistically significant ($p < 0.05$) (Purnomo *et al*., 2014; Purnomo *et al*., 2023).

4. RESULT AND DISCUSSION

4.1. Screening of DDT Tolerance Fungal Isolates

From a total of 40 DDT-tolerant fungal isolates, three fungal isolates that could grow at a very high DDT concentration (10,000 ppm) were screened, following a careful assay for further optimization procedures. These isolates were given designations as AS1, AS1T1, and T1. The finally-selected fungal isolates AS1 and T1 were used as co-inoculants after a compatibility test assessment, as shown in Fig. 7. This was done because they had the highest biomass production and sporulation efficacy. The two fungi were selected for potential DDT degradation. This final *in vitro* study was carried out using GC-ECD analysis.

The results of the DDT-amended media type showed greater tolerance index (TI) values than control media. A value of < 100% tolerance index was observed in fungal isolate T1, and a value >100% tolerance index (TI) was observed in fungal isolates AS1 and AS1T1, as illustrated in Table 2. This result revealed that the co-inoculated fungal isolates AS1T1 have a high capability to degrade DDT. The fungal isolate grown in media with pH 4, pH 7, and pH 10 values at 25, 30, and 35 °C indicates that the selected fungal isolates can tolerate a wide range of pH as well as temperature and were selected for DDT degradation at very high DDT concentrations, as shown in Tables 2 and 3.

A study conducted by Pandey *et al.* (2021) investigated how the biodegradation of DDTs was influenced by the addition of extra nutrient sources, growing conditions at various time intervals, temperatures, and pH. However, the current fungal co-culture AS1T1 was different from those microorganisms previously reported in the literature that could degrade DDT at high concentrations because the current co-cultured isolates can utilize DDT as a sole carbon source and show high tolerance towards very high DDT concentrations, alkaline and acidic medium, as well as a wide range of temperatures by synergetic interaction effect. This confirmed the capacity of fungal isolates co-culture AS1T1 to adapt a wide range of habitats sequentially, and it also proved that applying this fungal co-culture to the real environment might be an effective, low-energy, and sustainable way to remove DDT contaminants in environmental clean-up (Gupta *et al.*, 2014).

4.2. Morphological Identification of Fungal Isolates

All the finally selected fungal isolates displayed distinct sizes, shapes, colors, and textures on PDA plates following a 3–7 day incubation period. The morphological traits of all selected filamentous fungi differed from one another. The fungal isolate AS1 grew rapidly and formed loose colonies on PDA plates, with all selected *spp*ecies exhibiting characteristic colors and shapes.

The macroscopic observation of the fungal isolate AS1 obverse side growths appeared whitish but turned black with time on PDA plates, and the reverse side of the colony was colorless to light yellow (Table 1 and Fig. 6 (A-B)). The microscopic examination with lactophenol cotton blue staining, hyaline septate hyphae, and biseriate phialides covering the entire vesicle with radiate conidial heads was observed (Table 1 and Fig. 6 (C)).

The macroscopic observation of the fungal isolate T1 obverse side growths appeared white at an earlier stage but turned deep green with time on PDA plates, and the reverse side of the colony was yellowish (Table 6 (D-E)). The microscopic examination of fungal isolate T1 with lactophenol cotton blue staining shows that it is branched and organized in a pyramidal structure with longer branches at the base that progressively shorten as it nears the tip (Table 2 and Fig. 6 (F)).

Table 1

Morphological and microscopic characterization of fungal isolate AS1 and T1

Fungal isolate	**Macroscopic characteristics of the colony observation**				
AS1	**Surface colour**	**Margins**	**Reverse side**	**Elevations**	**Growth**
	Dark brown to black	Entire	Without colour	Umbonate	Rapid
T1	White at early stage	Entire	yellow		Rapid
	Later deep green				
Isolate	**Microscopic characteristics of the colony observation**				
AS1	**Hyphae**	**Conidiophore**	**Conidia**	**Phialides**	**Fruiting body**
	Branched	Length	Heads	Biseriate	Cleistothecia
	Septate	200 to 400µm	Blackish brown	Primary	Present
		Diameter	Diameter	20 to 30 µm	
		7 to 10 µm	2.5 to 4 µm	Secondary	
		Vesicle	Ornametation	40 to 60 µm	
		Globose	Exine *spp*iny		

T1	**Hyphae**	**Conidiophore**	**Conidia heads**	**Phialides**	**Fruiting body**
	Branched	Length	green, ellipsoidal	3-4 whorls	Perithecia
	Septate	9.0–9.5 µm	smooth	Primary	Present
		Diameter	Diameter	5.5-9.0 µm	
		Globose to	3.5–4.5 × 2.2 3.5 µm		
		Sub globose	Ornametation		
			ellipsoidal		

Microscopic and macroscopic characters of fungal isolate AS1 and T1 re*spp*ectively

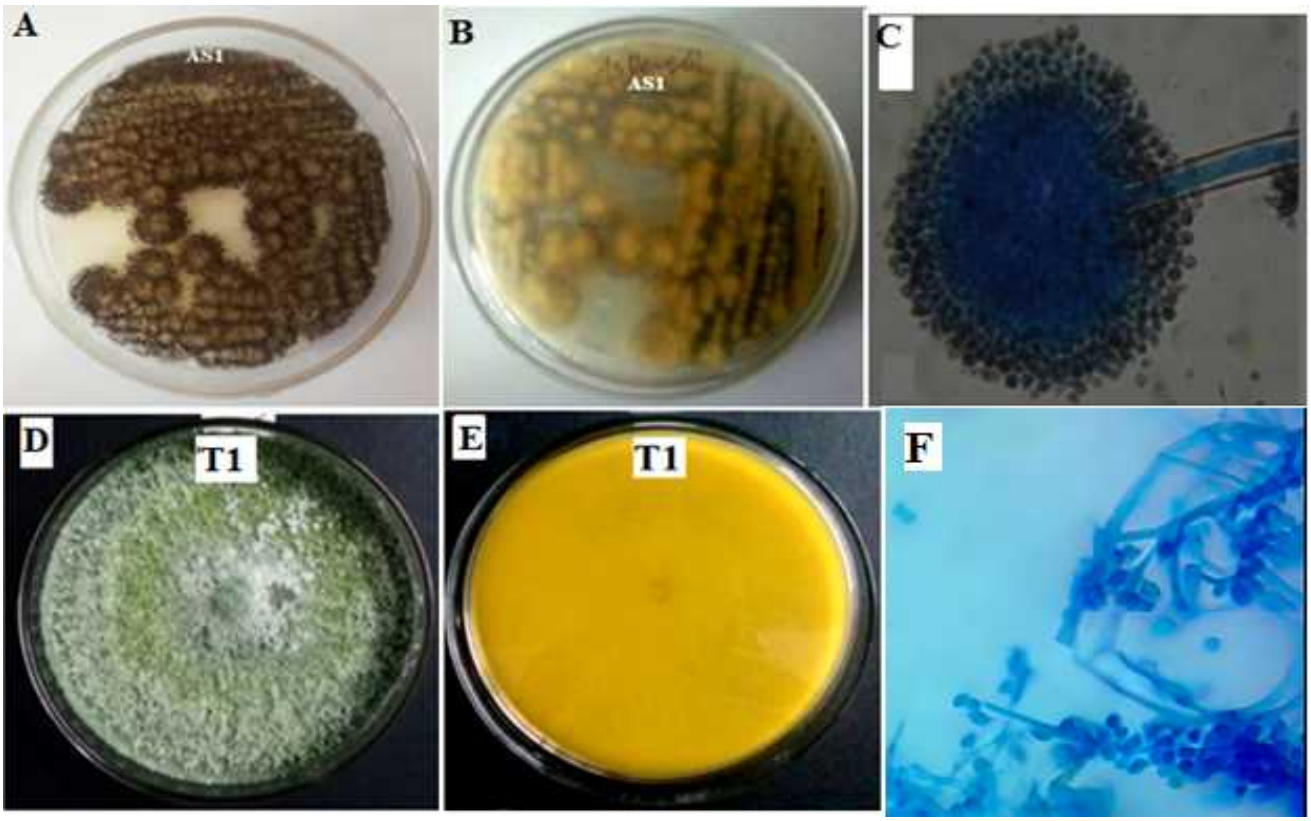


Fig. 6. (A) Macroscopic observation of isolate AS1 obverse side (B) Reverse side of AS1 (C) microscopic observation of isolate AS1(D)obverse side of isolate T1 (E) Reverse side of T1 (F)microscopic observation of isolate T1 (40X).

4.2.1. MALDI-TOF Mass Spectrometry Analysis

The last two selected fungal isolates, AS1 and T1, have been identified at the *spp*ecies level among three filamentous fungi. By using MALDI-TOF MS, the identification scores were ≥ 2.000 and mainly belonged to *A. niger and T. koningii*, respectively.

4.3. Compatibility Test among Fungal Isolates

The compatibility test was conducted on PDA culture plates to assess the interaction between the two potential DDT-degrading fungi, as illustrated in Fig. 7.A dominant fungus was almost covering the culture medium. The growth of fungal isolate T1 was found to be faster than that of AS1, so the former was covered onto the plate within 48 hours. The fungi were expanded toward one another throughout the three days of incubation at 25±2℃ until the meeting point, as indicated in Fig. 7. This compatibility test confirmed that the two fungi grow together in solid and liquid media and the two fungi were not showed antagonistic. These fungi may have a synergetic role in breaking down DDT and its metabolites. In mixed fungal co-cultures of DDT-amended media, each fungus may play a specific role in the breakdown of DDT. The intermediates produced by one fungus might use by another sequentially. When one of the fungi breaks down DDT into DDE and another metabolite that is more toxic than DDT, the other fungi has a synergistic role to detoxify or degrade this metabolite into other non-toxic byproducts. These two fungi may work in consortia and in succession, or sequentially, to break down DDT.

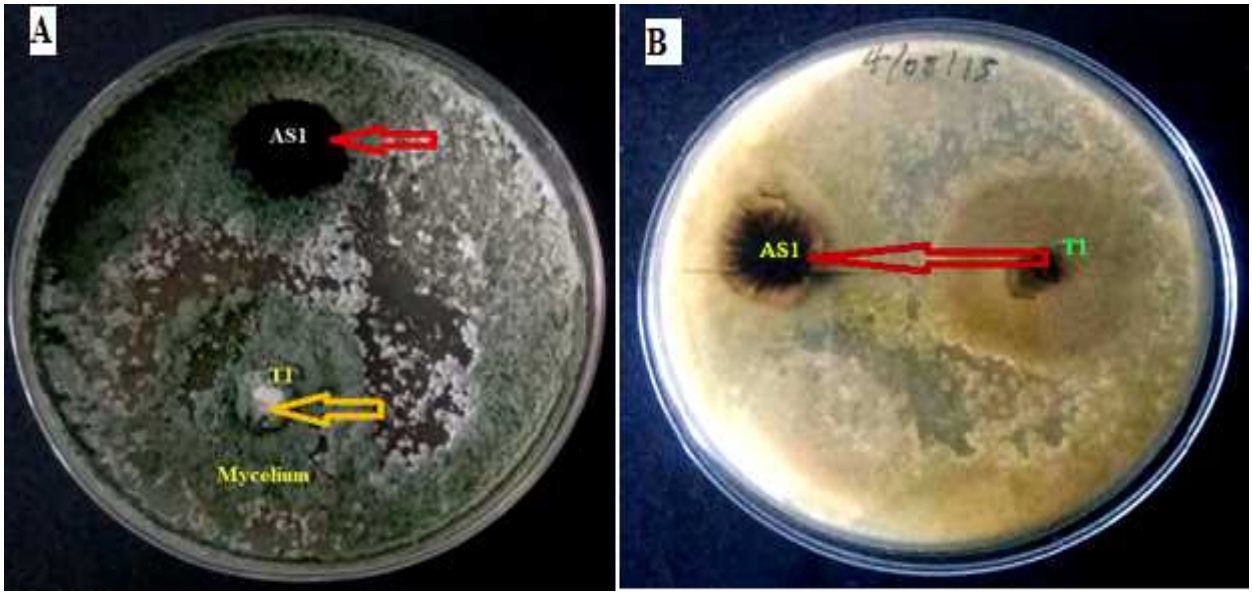


Fig.7. Hyphal interaction assay A) Obverse side of *A.niger*(isolate As1) and *T. koningii* (isolate T1) B) Reverse side of *A.niger*and *T. koningii*

4.4. Screening Significant Variables using One Way ANOVA Statistical Analysis for Determination of Fungal Biomass Production and Sporulation

The statistical ANOVA analysis results confirmed that the DDT concentration (0, 3500, and 7000 ppm) and the fungal isolates AS1, AS1T1, and T1 were the most significant factors for biomass production ($p<0.05$), as shown in Table 2. However, the concentrations of DDT (0, 3500, and 7000 ppm) had no statistically significant impact on sporulation ($p > 0.05$), as shown in Table 3. Temperature and pH had no significant impact on the production of fungal biomass and sporulation ($p > 0.05$), as indicated in Tables 2 and 3.

Table.2, Fungal growth parameters for biomass production carried out according to the Fisher test at different temperature, pH and DDT concentrations after 10 days incubation period

Fungal Isolates	Dry biomass in gram	TI %	DDT (ppm)	Dry biomass in gram	TI %	Temperature. ℃	Dry biomass in gram	TI %	pH	Dry biomass in gram	TI %
AS1T1	1.01±0.16[a]	153	7000	0.90±0.23[a]	136	35	0.88±0.32[a]	133	10	0.85±0.27[a]	129
AS1	0.94±0.14[a]	142	3500	0.88±0.17[ab]	133	25	0.87±0.29[a]	132	4	0.84±0.33[a]	127
T1	0.55±0.28±[b]	83	0	0.66±0.39[b]	100	30	0.78±0.27[a]	118	7	0.82±0.28[a]	124

The data represents the mean (±SD) of (n= 3) and the same lowercase letters indicate a lack of statistically significant differences ($p >0.05$) among means within the same column.

Table. 3, Fungal growth parameters for spore production carried out according to the Fisher test at different temperature, pH and DDT concentrations after 10 days incubation period

Fungal Isolate	Log spore/ mL	DDT (ppm)	Log spore/ mL	Temperature ℃	Log spore/ mL	pH	Log spore/ mL
TIASI	5.74±0.37[a]	0	5.65±0.36[a]	35	5.59±0.23[a]	4	5.61±0.40[a]
AS1	5.58±0.29[a]	3500	5.54±0.36[a]	25	5.54±0.44[a]	7	5.52±0.38[a]
T1	5.32±0.33[b]	7000	5.44±0.32[a]	30	5.52±0.44[a]	10	5.51±0.38[a]

The data represents the mean (±SD) of (n= 3) and the same lowercase letters indicate a lack of statistically significant differences ($p >0.05$) among means within the same column.

4.5. Statistical Optimization of biomass production and Sporulation for Identification of Significant Factors Using Response Surface Methodology (RSM)

The results of determined growth parameter affect the fungal biomass production and the sporulation efficacy, as shown in Tables 2, 3 and Fig.8 and Fig.9.The optimization result illustrates

that the highest dry biomass production was recorded by the co-inoculated fungal isolates AS1T1, which were grown in a 1:1 ratio of PDB + 7000 ppm DDT medium at 10.0 pH and 35°C during 10 days of continuous incubation period as seen in Fig. 10.

This finding showed that the two isolates, AS1 and AS1T1, produced biomass and sporulated in a similar way ($p > 0.05$), as shown in Tables 2 and 3. However, the isolate T1's capacity to form spores and biomass is significantly influenced by the various concentrations of DDT, pH, and temperature ($p < 0.005$), as indicated in Tables 2 and 3. The exposure to the various pH values and temperatures had little to no impact on the three chosen fungal isolates. Tables 2 and 3 show that fungal isolates grown on PDB media as a control produced significantly less dry biomass ($p < 0.005$) than the isolates exposed to medium containing DDT. The fungal isolates exposed to DDT at initial concentrations of 0, 3,500, and 7000 ppm did not significantly influence the efficacy of sporulation. ($p > 0.005$) (Table 3).

The maximum mean fungal dry biomass (1.01 g ± 0.37) was recorded in AS1T1 isolates. The maximum mean fungal spore production (5.74 log *sp*ores/mL) was recorded in AS1T1 isolates as shown in Table 2 and Figs. 11 and 12, cultured in control (PDB) media type, followed by a 1:1 ratio of PDB + 3500 ppm DDT media type at pH 4.0 and temperature 35 ± 2℃.

4.5.1. The Main and Interaction Effect Analysis between Influencing Factors

The ability to produce dry biomass and spores was not significantly different between the two fungal isolates, AS1 and AS1T1 ($p > 0.05$), as indicated in Table 2, but all selected factors could simultaneously influence dry biomass production and the sporulation capability of fungal isolate T1 significantly ($p < 0.05$), as shown in Tables 2 and 3. When compared to the control (PDB) media type with a 1:1 ratio (PDB + DDT) with 3500 and 7000 ppm DDT concentrations provided with mineral salt medium (MSM), the fungal dry biomass production of the control media type showed significant differences ($p < 0.05$) as indicated in Table 2. However, the media type 1:1 ratio of PDB + DDT and 3500 and 7000 ppm DDT concentrations significantly influenced sporulation capability ($p < 0.05$), as indicated in Table 3. The difference between pH-4.0 and 7.0, as well as pH-10 medium and temperature (20, 25, and 35℃), did not significantly influence dry biomass production and the sporulation capability ($p > 0.001$), as indicated in Tables 1 and 2.

Depending on the parameter, the statistical analysis of two-factor interactions (fungal isolate vs. DDT concentration), (DDT concentration vs. DDT concentration), (DDT concentration vs. pH), and (temperature vs. temperature) proved that these variable interactions significantly influenced fungal biomass production ($p < 0.05$), as shown in Fig. 8. However, the interactions between (pH vs. temperature), (fungal isolates vs. pH), (fungal isolates vs. temperature), (pH vs. pH), and (temperature vs. DDT concentration) did not significantly influence fungal biomass production ($p > 0.05$), as shown in Fig. 8. The various growth parameters, including the fungal isolates (AS1, AS1T1, and T1), the 1:1 ratio PDB enriched with DDT concentrations of (0, 3500, and 7000 ppm), and the temperatures (25, 30, and 35℃), proved that these variable interactions did not influence the efficacy of fungal sporulation ($p<0.05$) as shown in Fig.9.

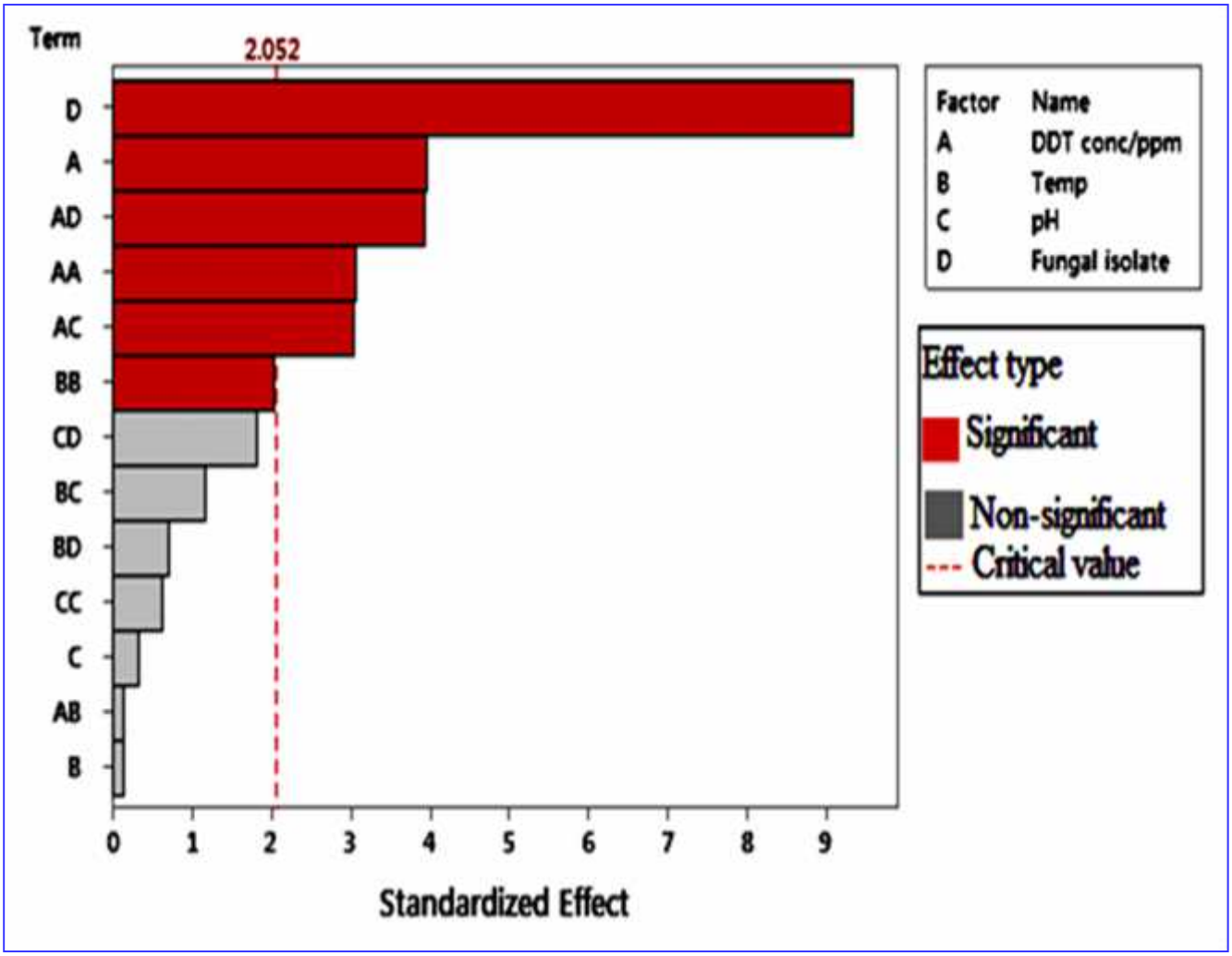


Fig.8. Pareto-regression graph indicating the effect of growth factors on biomass production.

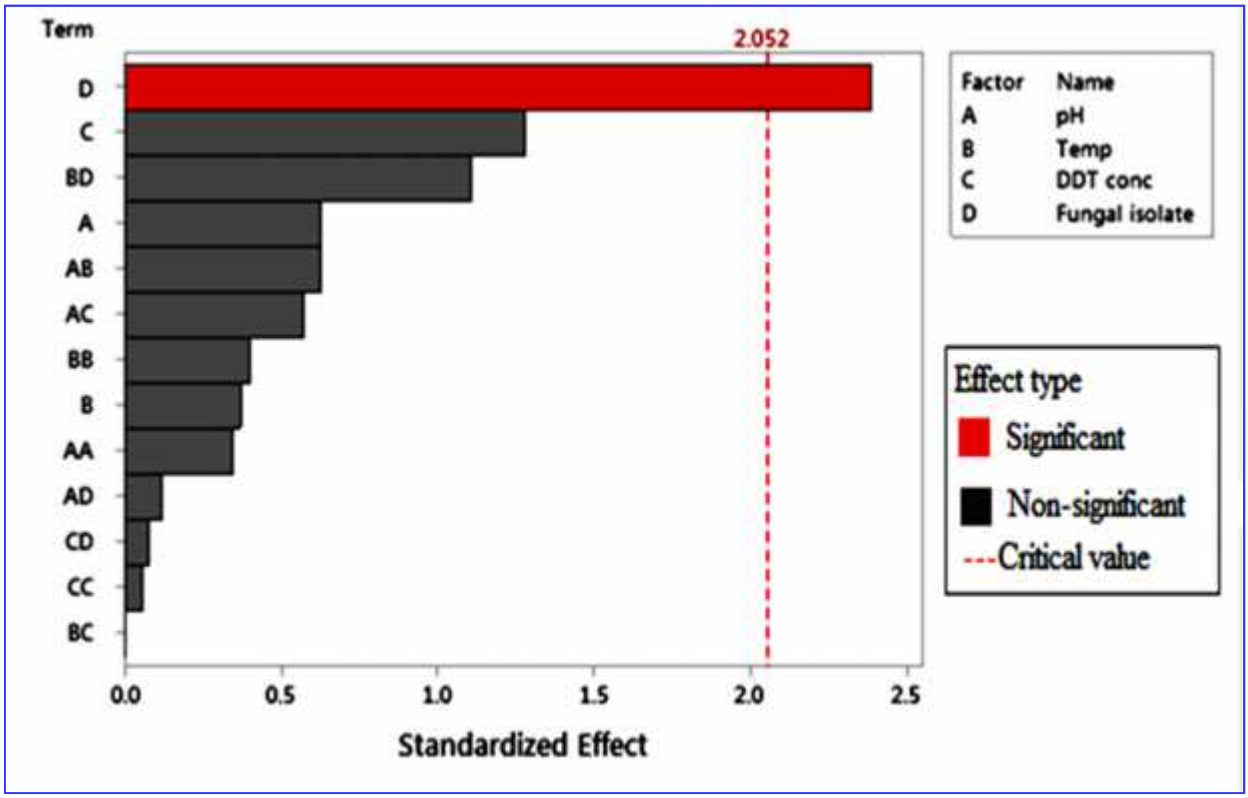
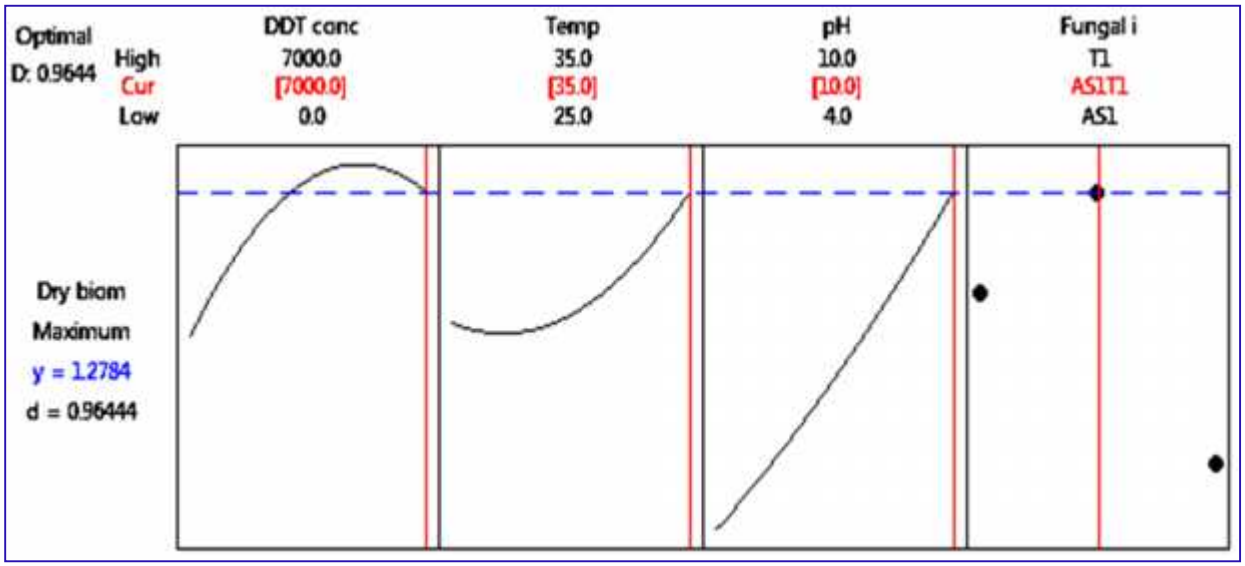
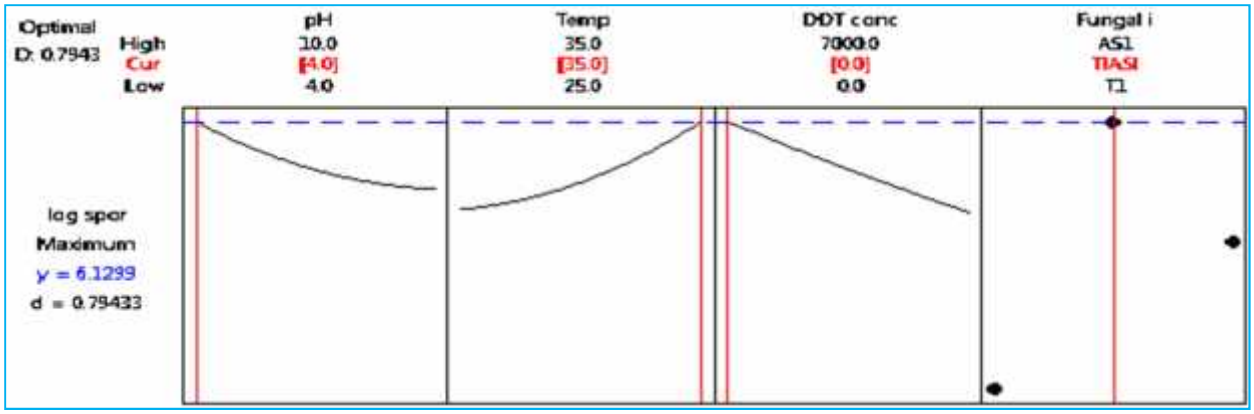


Fig.9. Pareto-regression graph indicating the effect of growth factors on *spores* production.



Fg.10.Optimization plot of dry biomass



Fg. 11.Optimization plot of *spores* production with controla media type

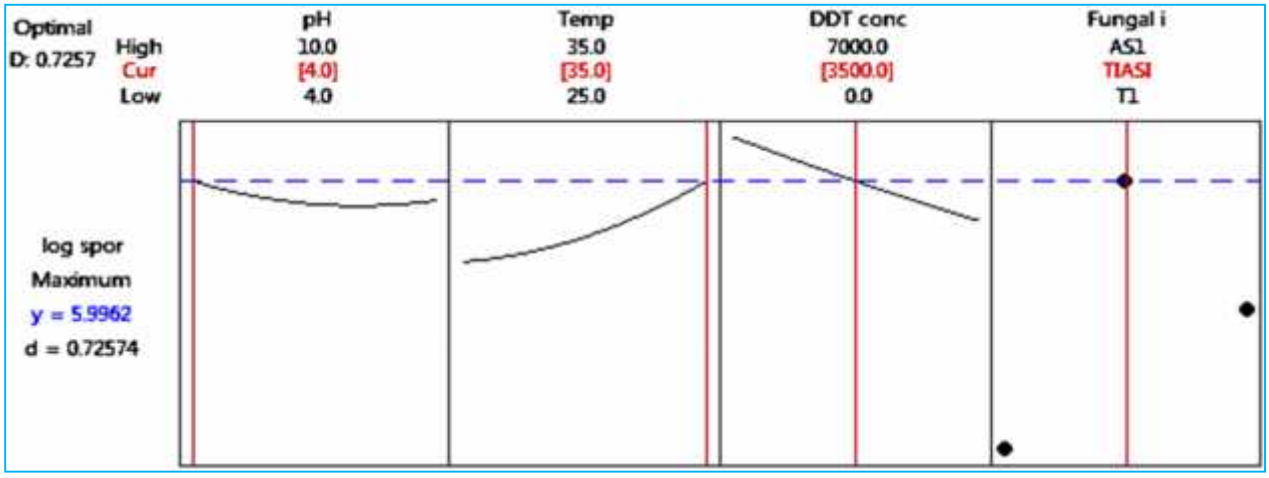


Fig. 12.Optimization plot of *spores* production without controla media type

4.6. DDT Degradation assay by Fungal *A. niger* and *T. koningii*

The culture medium with various DDT concentration extractions was analyzed using GC-ECD by comparing the retention time and electron capture of their constituents with reference samples in order to evaluate the rate of degradation of DDT by the fungal co-culture of *A. niger* and *T. koningii*. Three metabolites with retention times of 18.003, 19.411, and 20.666 min were identified from culture media extractions. They were identified as 1, 1-Dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE), 1, 1-Trichloro-2,2-bis (4-chlorophenyl) ethane (DDD), and 1, 1-Trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), respectively. This result is similar to a study by Purnomo *et al.* (2008), showing that a strain of brown rot fungi produced DDE, DDD, and DBP from DDT degradation.

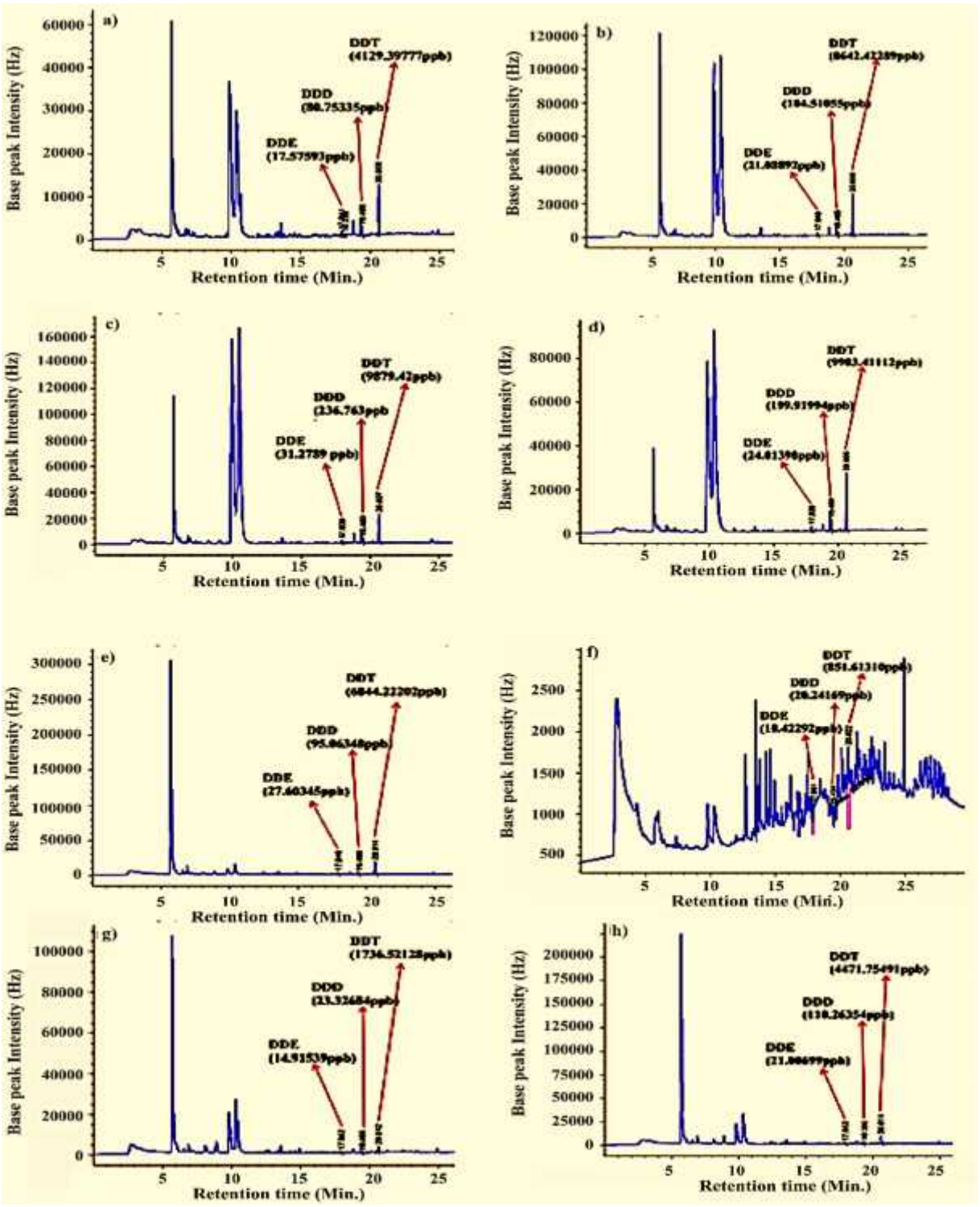


Fig. 13. Gas chromatogram of DDT degradation by mixed culture *A.niger*and *T.Koningii* after 15 days cultured (supplied with 1750, 3500, 5250 and 7000ppmDDT): (a, b, c and d) electron capture of product extracted from 100% pure DDT fungal mycelia and (e, f, g, and h) extracted from 1:1 ratio of PDB + DDT fungal mycelia respectively.

Table. 4, Percentage residual and degradation analysis of DDT by fungal co-culture *A.niger*and *T. koningii* in liquid (MSM) cultures during a 15-days inoculation period detected by GC-ECD

	% residual analysis			
Initial DDT (ppm)	1750	3500	5250	7000
Media types				
100% DDT	0.003±0.003[a]	0.083±0.141[a]	0.064±0.107[a]	0.002±0.002[a]
Enriched DDT	0.005±0.003[a]	0.001±0.000[a]	0.478±0.22[b]	0.001±0.001[a]
	%Degradation			
100% DDT	99.997±0.003[a]	99.917±0.141[a]	99.936±0.107[a]	99.998±0.002[a]
Enriched DDT	99.995±0.003[a]	99.999±0.000[a]	99.522±0.22[b]	99.999±0.001[a]

The data represents the mean (±SD) (n= 3) and the same lowercase letters indicate a lack of statistically significant differences ($p < 0.05$) among means within the same row.

After 15 days of addition of 10^8 spores/mL of fungal co-culture *A. niger* and *T. koningii* into DDT amended media, only 0.003 ppb, 0.083 ppb, 0.064 ppb, and 0.002 ppb were found in 100% pure DDT media, and 0.005 ppb, 0.001 ppb, 0.478 ppb, and 0.001 ppb in the 1:1 ratio PDB + DDT media of mean percentage residual analysis were found, as shown in Table 4 and Figs. 13 (a-h). The GC-ECD result analysis confirms that the synergistic interactions between the two fungi have caused DDT to almost completely disappear from both culture mediums when compared to the initial DDT concentration of 1750, 3500, 5250, and 7000 ppm, respectively.

Table. 5, Percentage residual and degradation analysis of DDT and its metabolic products by fungal co-culture *A.niger*and *T. koningii* in liquid (MSM) cultures during a 15-days inoculation period detected by GC-ECD

Metabolic products	DDT	DDD	DDE
(%) Residual	0.146±0.249[a]	0.058±0.154[a]	0.036±0.099[a]
% degradation	99.854±0.249[a]	99.942±0.154[a]	99.964±0.099[a]

The data represents the mean (±SD) (n= 3) and the same lowercase letters indicate a lack of statistically significant differences ($p < 0.05$) among means within the same row.

Very small amounts of DDT, DDD, and almost no DDE were found in extracts of all media. After 15 days of shaker incubation at 27°C and 120 rpm, the mean metabolic products for DDT, DDD, and DDE were calculated from the initial concentration at 1750, 3500, 5250, and 7000 ppm DDT, as shown in Table 5 and Figs. 13 (a-h). The DDT concentrations in liquid cultures were higher than those in DDE and DDD, suggesting that DDT is the main residues of DDT degradation in mixed cultures of *A. niger* and *T. koningii*, which is similar to the research of Bumpus and Aust (1987) on DDT degradation by white rot fungi and *P. chrysospporium*. The current GC-ECD result analysis clearly indicates that the fungal co-culture of *A. niger* and *T. koningii* almost completely degraded DDT and its metabolites (DDD and DDE) sequentially, as shown in Table 5. As far as reviewed different literature and knowledge goes, this study could be the first case reporting the complete degradation of DDT by the synergetic interaction of fungal isolates in very high DDT concentrations up to 7000 ppm.

Although these isolates have a unique trait for DDT degradation capability, it needs rigorous study to determine if it is accompanied by genetic factors or not since it was isolated from a polluted tropical environment surrounding industries. Table 5 and Figs. 13 (a-h) show that after being co-cultured at extremely high DDT concentrations for 15 days, 99.854% of DDT, 99.942% of DDD, and 99.964% of DDE had been eliminated from the culture media. These results revealed that the very high capability of the fungal co-cultures *A. niger* and *T. koningii* for DDT and its main metabolite degradation at very high DDT concentrations.

According to a study conducted by Russo. (2019), a number of fungi have the ability to degrade DDT. According to the study conducted by Al-Rashed. (2021), the marine bacterium *Paracoccus spp*. DDT-21 rapidly detoxified DDT up to 50 mg/L. The study by Santacruz *et al*. (2005) found that about 99% of DDT was removed from the high DDT concentration (150 ppm). However, a recent study confirmed that *A. niger* and *T. koningii* mixed cultures are different from those microorganisms previously reported; the *A. niger* and *T. koningii* co-cultured in liquid media supplied with a very high DDT concentration (7000 ppm) and incubated at a temperature of 27±2℃ for 15 days on a shaker revealed that 99.9–99.99% of DDT disappeared from the culture medium, as illustrated in Table 4 and Fig. 13 (a-h). According to the study conducted by Boelan *et al*. (2019), the co-culture of *G. lingzhi* and *P. aeruginosa* degraded DDT up to 100 ppm, while *G. lingzhi* degraded up to 52.52 ppm after a 7-day incubation period in the PDB medium. The

study conducted by Boelan *et al*. (2018), the co-culture of WRF *Ganoderma lingzhi* and *B. subtilis* bacterium degraded DDT up to about 82.30 ppm. The removal of 99.9–99.99% of DDT and its main metabolites from culture media in a wide range of growth factors may confirmed that the applications of fungal co-culture *A. niger* and *T. koningii* have the potential to degrade and cleanup DDT pesticide pollutants from the environment successfully.

The finding of this study also confirmed that increasing initial DDT concentrations from 1750, 3500, 5250, to 7000 ppm in both 1:1 ratio (PDB + DDT) and 100% pure DDT amended media did not significantly influence ($p > 0.05$) the fungal degradation efficacy of the selected fungal co-cultured *A. niger* and *T. koningii* except 5250 ppm DDT concentration as shown in Table 4. The ANOVA statistical analysis of GC-ECD results confirmed that DDT and its metabolic products, DDD and DDE, were almost completely removed without showing significant differences among them ($p > 0.05$), as indicated in Table 5. Fungal co-culture *A. niger* and *T. koningii* transforms DDT to DDE via dehydrochlorination, followed by DDE reductive dechlorination. In this study, the proposed DDT degradation pathway by fungal co-culture *A. niger* and *T. koningii* is shown in Fig. 14. This finding also revealed the very high capability of synergetic interaction effects of the screened fungal co-culture *A. niger* and *T. koningii* to degrade DDT and its main metabolic byproducts in succession, making them an effective and promising candidate for mycorrhizal environmental application purposes.

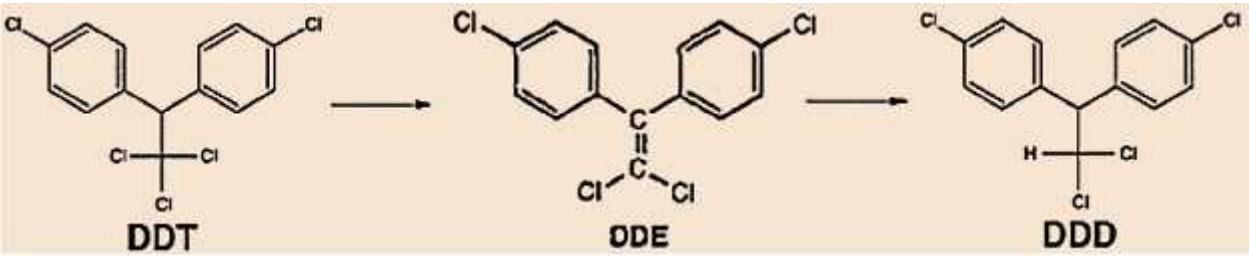


Fig. 14.The proposed pathway for the myco-degradation of DDT by mixed culture *A.niger*and *T. koningii*

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The long-term accumulation and aging of DDT and its residues in the environment results in additional constraints on treating contaminated sites, wildlife, and human health. The fungal isolates capable of degrading DDT and its main residues (DDE and DDD) AS1 and T1 were identified as *A. niger* and *T. koningii*. The optimization results revealed that the co-cultured fungal isolates, *A. niger* and *T. koningii*, produced the highest biomass and spores after 10 days of incubation at 35℃ with pH 10, and pH 4. These fungal isolates could degrade DDT up to 7000 ppm, supplied with MSM efficiently. The high concentrations of DDT (3500 and 7000 ppm) have no toxic effect on the fungal biomass production or sporulation capability. It was found to contain DDT, DDD, and DDE, confirming a decrease in DDT and its metabolite degradation without exhibiting significant differences, respectively ($p>0.05$). A very high mean degradation of GC-ECD analysis (99.796%) was observed at DDE. Therefore, the mixed cultures of the fungal isolates *A. niger* and *T. koningii* are a promising candidate for the removal or detoxification of DDT and its degrading metabolites from a polluted environment by synergetic interaction effect. It could also be used for biofermentation of DDT for disposal purposes to avoid the high expense to the countries for absolute DDT pesticide disposal.

Recent *in vitro* studies have evaluated fungal additives that may increase bioavailability and degrade DDT by synergetic interactions in long-term DDT-contaminated environments. The degradation potential of fungal co-cultures *A. niger* and *T. koningii* for DDT pesticides under liquid media conditions plays a more efficient role in the bioaugmentation of fungal consortiums. Although DDT is one of the most difficult contaminants to degrade, recent advances have created new avenues in *in vitro* complete degradation of DDT and its residues up to 7000 ppm DDT concentration by fungal co-culture *A. niger* and *T. koningii.* So far, no study has been able to provide insights into microbial DDT degradation with such an amount of DDT concentration. Furthermore, this *sp*ecies can efficiently survive under a wide range of harsh and suitable environmental conditions.

5.2. Recommendation

From the results of this study, the following recommendations are suggested

- Further studies still need to mineralize DDT completely in real environmental conditions, such as the whole environmental microbial metagenomics analysis and fungal tolerance study against several environmental pollutants such as heavy metal, azo dye, PAH, etc.
- This fungal co-culture needs to explore the effect of non-axenic conditions on artificial fungal-fungal co-cultures, whether they outcompete indigenous microbes in environmental matrices or not.
- The field applications ought to be conducted in various places.
- Further study also should be done to increase the transgenic microorganisms' survivability when released in the environment, as their detoxification and breakdown of DDT contaminants have been greatly enhanced.
- Environmental conditions for effective and improved microorganism growth, DDT degradation operating parameters, and DDT bioremediation process mechanisms must all be rigorously analyzed.
- Enzyme systems that produce the best yields and efficiency with the least amount of water, energy, and nutrients need to be developed.
- Even though genetic engineering methods produce more enzymes with greater efficiency, more investigation is required to tackle the genetic engineering method's financial challenge.
- A stakeholder targeting environmental pollution in the overall parts of Ethiopia enhances the success of ongoing DDT-polluted ecosystem treatment initiatives.
- Based on these research findings, policymakers should receive a policy brief on environmental contamination caused by banned POPs, such as DDT and its bioremediation mechanisms for further research.

References

Abbasi, E., Vahedi, M., Bagheri, M., Gholizadeh, S., Alipour, H., & Moemenbellah-Fard, M. D. (2022). Monitoring of synthetic insecticides resistance and mechanisms among malaria vector mosquitoes in Iran: A systematic review. *Heliyon*, *8*(1).

Abdel Gawad, H., & Abdelhameed, R. (2023). Removal Methods of Pesticides and Their Improvement Using Metal-Organic Frameworks. *Egyptian Journal of Chemistry*.

Abeshu, M. A., Lelisa, A., & Geleta, B. (2016). Complementary feeding: review of recommendations, feeding practices, and adequacy of homemade complementary food preparations in developing countries–lessons from Ethiopia. *Frontiers in nutrition*, *3*, 41.

Abioye, O. P., Ijah, U. J. J., Aransiola, S. A., Auta, S. H., & Ojeba, M. I. (2021). Bioremediation of toxic pesticides in soil using microbial products. *Mycoremediation and Environmental Sustainability: Volume 3*, 1-34.

Aggarwal, S., & Kumari, A. (2024). Role of fungi in biotechnology. In *Entrepreneurship with Microorganisms* (pp. 39-67). Academic Press.

Ahmed, S. F., Mofijur, M., Nuzhat, S., Chowdhury, A. T., Rafa, N., Uddin, M. A., & Show, P. L. (2021). Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. *Journal of hazardous materials*, *416*, 125912.

Ahmad, T., Rafatullah, M., Ghazali, A., Sulaiman, O., Hashim, R., & Ahmad, A. (2010). Removal of pesticides from water and wastewater by different adsorbents: a review. *Journal of Environmental Science and Health, Part C*, *28*(4), 231-271.

Akpan, G. E., Ndukwu, M. C., Etim, P. J., Ekop, I. E., & Udoh, I. E. (2023). Food Safety and Agrochemicals: Risk Assessment and Food Security Implications. In *One Health Implications of Agrochemicals and their Sustainable Alternatives* (pp. 301-333). Singapore: Springer Nature Singapore.

Alemu, Z. G., Oosthuizen, K., & Van Schalkwyk, H. D. (2003). Contribution of agriculture in the Ethiopian economy: A time-varying parameter approach. *Agrekon*, *42*(1), 29-48.

Al-Rashed, S., Marraiki, N., Syed, A., Elgorban, A. M., Prasad, K. S., Shivamallu, C., & Bahkali, A. H. (2021). Bioremediation characteristics, influencing factors of dichlorodiphenyltrichloroethane (DDT) removal by using non-indigenous Paracoccus *spp*. *Chemosphere*, *270*, 129474.

Alvarez, P. J., Metz, J., Javed, H., & Mathieu, J. (2021). SERDP Project ER20-1430.

Amutova, F., Jurjanz, S., Akhmetsadykov, N., Kazankapova, M., Razafitianamaharavo, A., Renard, A., & Delannoy, M. (2023). Adsorption of organochlorinated pesticides: adsorption kinetic and adsorption isotherm study. *Results in Engineering*, *17*, 100823.

Anju, A., Ravi S, P., & Bechan, S. (2010). Water pollution with special reference to pesticide contamination in India. *Journal of water resource and protection*, *2010*.

Ashine, E. T., Tadesse Bedane, M., & Asefa Mengesha, A. (2024). Response of Carrot (Daucus carota L.) to Supplementary Irrigation under Rain-Fed Agriculture at Jimma and Gera, Jimma Zone, South West Ethiopia. *Advances in Agriculture*, *2024*.

Ateş, A. (2019). *Cost of reducing micropollutant load from urban wastewater treatment plants in Turkey* (Master's thesis, Middle East Technical University).

Aziz, N., Craig, P., Mirzaghorbanali, A., Rasekh, H., Nemcik, J., Li, X. (2015). Behaviour of cable bolts in shear; experimental study and mathematical modelling.

Bakshi, A.D. Singh, J. Kour, S. Jan, M. Ibrahim, B.A. Mir, Advanced Technologies for the Remediation of Pesticide-Contaminated Soils. Handbook of Assisted and Amendment: Enhanced Sustainable Remediation Technology. (2021) 331-53,

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S., & Tripathi, M. (2022). Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. *Toxics*, *10*(8), 484.

Barau, B. W., Yusufu, W. N., Abubakar, A., & Fadilah, A. S. (2023). Pesticide Residue Contamination of Some Cereals and their Consequential Health Implication in the Food Chain of Taraba Northern Geo-political Region, Taraba State. *Asian Journal of Environment & Ecology*, *22*(1), 1-18.

Barragan-Huerta, B. E., Costa-Pérez, C., Peralta-Cruz, J., Barrera-Cortés, J., E*spp*arza-García, F., & Rodríguez-Vázquez, R. (2007). Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. *International Biodeterioration & Biodegradation*, *59*(3), 239-244.

Barrón Cuenca, J., Dreij, K., & Tirado, N. (2024). Human pesticide exposure in Bolivia: a scoping review of current knowledge, future challenges and research needs. *International journal of environmental research and public health*, *21*(3), 305.

Baxter, J., & Cummings, S. P. (2008). The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil. *Journal of applied microbiology*, *104*(6), 1605-1616.

Beard, J., & Australian Rural Health Research Collaboration. (2006). DDT and human health. *Science of the total environment*, *355*(1-3), 78-89.

Bedine, M.A.B., Taïeb, N., Agriopoulou, S., Miché, L., Moussango, D., Sameza, M.L., Dupuy, N., Roussos, S., Fekam Boyom, F. (2022). Identification of native soil-derived Trichoderma *spp*. isolates and analysis of their antagonist traits against *Lasiodiplodia theobromae* causing stem-end rot in papaya. *Archives of Phytopathology and Plant Protection*, *55*(15), pp.1766-1794.

Behera, S., & Das, S. (2023). Potential and prospects of Actinobacteria in the bioremediation of environmental pollutants: Cellular mechanisms and genetic regulations. *Microbiological Research*, 127399.

Bello, S., Pérez, N., Kiebist, J., Scheibner, K., Ruiz, M. I. S., Serrano, A., & Moreira, M. T. (2021). Early-stage sustainability assessment of enzyme production in the framework of lignocellulosic biorefinery. *Journal of Cleaner Production*, *285*, 125461.

Bhatt, P., Bhatt, K., Sharma, A., Zhang, W., Mishra, S., & Chen, S. (2021). Biotechnological basis of microbial consortia for the removal of pesticides from the environment. *Critical Reviews in Biotechnology*, *41*(3), 317-338.

Bisht, J., Harsh, N.S.K., Palni, L.M.S., Agnihotri, V. and Kumar, A. (2019). Biodegradation of chlorinated organic pesticides endosulfan and chlorpyrifos in soil extract broth using fungi. *Remediation Journal*, *29*(3), pp.63-77.

Blachnio, M., Kusmierek, K., Swiatkowski, A. and Derylo-Marczewska, A. (2023). Waste-Based Adsorbents for the Removal of Phenoxyacetic Herbicides from Water: A Comprehensive Review. *Sustainability*, *15*(23), p.16516.

Boelan, E. G., & Purnomo, A. S. (2018). Abilities of co-cultures of white-rot fungus Ganoderma lingzhi and bacteria Bacillus subtilis on biodegradation DDT. In *Journal of Physics: Conference Series* (Vol. 1095, No. 1, p. 012015). IOP Publishing.

Boelan, E. G., & Purnomo, A. S. (2019). Biodegradation of 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) by mixed cultures of white-rot fungus Ganoderma lingzhi and bacterium Pseudomonas aeruginosa. *HAYATI Journal of Biosciences*, *26*(2), 90-90.

Bokade, P., Gaur, V. K., Tripathi, V., Bobate, S., Manickam, N., & Bajaj, A. (2023). Bacterial remediation of pesticide polluted soils: exploring the feasibility of site restoration. *Journal of Hazardous Materials*, *441*, 129906.

Bolor, V.K., Boadi, N.O., Borquaye, L.S., Afful, S., 2018. Human risk assessment of organochlorine pesticide residues in vegetables from Kumasi, Ghana. *J Chem* 2018:1–11.

Bose, S., Kumar, P. S., Vo, D. V. N., Rajamohan, N., & Saravanan, R. (2021). Microbial degradation of recalcitrant pesticides: a review. *Environmental Chemistry Letters*, *19*, 3209-3228.

Brovini, E. M., Moreira, F. D., Martucci, M. E. P., & de Aquino, S. F. (2023). Water treatment technologies for removing priority pesticides. *Journal of Water Process Engineering*, *53*, 103730.

Bumpus, J. A., & Aust, S. D. (1987). Biodegradation of environmental pollutants by the white rot fungus Phanerochaete chryso*spp*orium: involvement of the lignin degrading system. *BioEssays*, *6*(4), 166-170.

Calle, E. E., Frumkin, H., Henley, S. J., Savitz, D. A., & Thun, M. J. (2002). Organochlorines and breast cancer risk. *CA: a cancer journal for clinicians*, *52*(5), 301-309.

Cárdenas-Moreno, Y., González-Bacerio, J., García Arellano, H., & del Monte-Martínez, A. (2023). Oxidoreductase enzymes: Characteristics, applications, and challenges as a biocatalyst. *Biotechnology and Applied Biochemistry*, *70*(6), 2108-2135.

Castro-Vargas, M. S., & Werner, M. (2023). Regulation by impasse: Pesticide registration, capital and the state in Costa Rica. *Environment and Planning E: Nature and Space*, *6*(2), 901-922.

Cavalier, H., Trasande, L., & Porta, M. (2023). Exposures to pesticides and risk of cancer: evaluation of recent epidemiological evidence in humans and paths forward. *International Journal of Cancer*, *152*(5), 879-912.

Ceci, A., Pinzari, F., Russo, F., Persiani, A.M., Gadd, G.M. (2019). Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. *Applied microbiology and biotechnology*, *103*, pp.53-68.

Ceci, A., Rhee, Y.J., Kierans, M., Hillier, S., Pendlowski, H., Gray, N., Persiani, A.M., Gadd, G.M. (2015). Transformation of vanadinite [Pb5 (VO4) 3Cl] by fungi. *Environmental Microbiology*, *17*(6), pp.2018-2034.

Centers for Disease Control and Prevention. (2009). Fourth national report on human exposure to environmental chemicals.

Chala, M. (2022). Review of pesticide use in vegetable farms and its consequences in Ethiopia's central rift valley. *Journal of Agricultural Research Pesticides and Biofertilizers*, *3*(3).

Chan, S. S., Khoo, K. S., Chew, K. W., Ling, T. C., & Show, P. L. (2022). Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium-A review. *Bioresource Technology*, *344*, 126159.

Chang, J. H., Kumar, M., & Shen, S. Y. (2022). Agricultural application of visible light photocatalyst. In *Nanostructured Materials for Visible Light Photocatalysis* (pp. 467-489). Elsevier.

Chaturvedi, M., Joy, S., Gupta, R. D., Pandey, S., & Sharma, S. (2023). Endocrine disrupting chemicals (EDCs): chemical fate, distribution, analytical methods and promising remediation strategies–a critical review. *Environmental Technology Reviews*, *12*(1), 286-315.

Chaudhari, Y. S., Kumar, P., Soni, S., Gacem, A., Kumar, V., Singh, S., & Yadav, K. K. (2023). An inclusive outlook on the fate and persistence of pesticides in the environment and integrated eco-technologies for their degradation. *Toxicology and Applied Pharmacology*, 116449.

Chattopadhyay, S., & Chattopadhyay, D. (2015). Remediation of DDT and its metabolites in contaminated sediment. *Current Pollution Reports*, *1*(4), 248-264.

Chaukura, N., Muzawazi, E. S., Katengeza, G., & Mahmoud, A. E. D. (2022). Remediation technologies for contaminated soil systems. In *Emerging Contaminants in the Terrestrial-Aquatic-Atmosphere Continuum:* (pp. 353-365). Elsevier.

Chen, W. H., Biswas, P. P., Kwon, E. E., Park, Y. K., Rajendran, S., Gnanasekaran, L., & Chang, J. S. (2023). Optimization of the process parameters of catalytic plastic pyrolysis for oil production using design of experiment approaches: A review. *Chemical Engineering Journal*, 144695.

Choudhury, P. P. (2019). Biodegradation of topramezone by a Trichoderma isolate in soil. *Weeds-Journal of the Asian-Pacific Weed Science Society*, *1*(1), 43-54.

Chugh, M., Kumar, L., & Bharadvaja, N. (2022). Fungal diversity in the bioremediation of toxic effluents. In *Development in wastewater treatment research and processes* (pp. 61-88). Elsevier.

Clarke, S., & Lean, T. (2022). Turning DDT into 'Didimac': Making insecticide products and consumers in British farming after 1945. *History and Technology*, *38*(1), 31-61.

Cui, N., Pan, X., & Liu, J. (2024). Distribution, sources and health risk assessment of DDT and its metabolites in agricultural soils in Zhejiang Province, China. *Environmental Technology*, *45*(8), 1522-1530.

Cui, N., Pan, X., Liu, J. (2022). Distribution, sources and health risk assessment of DDT and its metabolites in agricultural soils in Zhejiang Province, China. *Environmental Technology*, pp.1-9.

Dad, K., Zhao, F. L., Hassan, R., Javed, K., Nawaz, H., Saleem, M. U., & Nawaz, M. (2022). Pesticides uses, impacts on environment and their possible remediation strategies-a review.

Dar, M.A., Shahnawaz, M., Hussain, K., Gupta, P., Sirwal, M.Y., Sadaqat, B., Gazal, S., Akhtar, R., Parihar, S., Zhu, D. and Adetunji, C.O. (2023). Natural compounds for bioremediation and biodegradation of pesticides. In *New Horizons in Natural Compound Research* (pp. 445-488). Academic Press.

Dardiotis, E., Aloizou, A. M., Sakalakis, E., Siokas, V., Koureas, M., Xiromerisiou, G., & Hadjigeorgiou, G. M. (2020). Organochlorine pesticide levels in Greek patients with Parkinson's disease. *Toxicology reports*, *7*, 596-601.

de Andrade, V. S., & de Mattos, M. C. (2023). Chloroaromatics: Advances in Synthesis and Applications. *Industrial Arene Chemistry: Markets, Technologies, Sustainable Processes and Cases Studies of Aromatic Commodities*, *4*, 1769-1802.

de Melo, D. W., Fernandez-Lafuente, R., & Rodrigues, R. C. (2023). Enhancing biotechnological applications of l-asparaginase: Immobilization on amino-epoxy-agarose for improved catalytic efficiency and stability. *Biocatalysis and Agricultural Biotechnology*, *52*, 102821.

Derouiche, A., Achour, A., & Driss, M. R. (2023). Organochlorine pesticides and polychlorinated biphenyls in raw bovine milk from various dairy farms in Beja, Tunisia: contamination status, dietary intake, and health risk assessment for the consumers. *Environmental Science and Pollution Research*, *30*(24), 65427-65439.

Dewangan, S., Bhatia, A. K., Singh, A. K., & Carabineiro, S. A. (2021). Removal of hydrophobic contaminants from the soil by adsorption onto carbon materials and microbial degradation. *C*, *7*(4), 83.

Dhanaraj, C. J. (2024). Harmful Effects of Water Pollution. *Handbook of Water Pollution*, 123-148.

Domsch, K., Gams, W., Anderson, T.H., 2007. Compendium of soil fungi. *IHW-Verlag, Eching*.

Donham, K. J., & Thelin, A. (2016). Health effects of agricultural pesticides. *Agricultural Medicine: Rural Occupational and Environmental Health, Safety, and Prevention*, *205*.

Douglas, D., & Douglas, K. (2019). Firefighter chemical Review-extension to review additional chemical substances–arp1701.

Doukani, K., Boukirat, D., Boumezrag, A., Bouhenni, H., & Bounouira, Y. (2022). Fundamentals of biodegradation process. In *Handbook of biodegradable materials* (pp. 1-27). Cham: Springer International Publishing.

e Rocha, N. L. T., Vieira, P. R., Goldenstein, J. P. N., Santiago, E. P., & Bassin, J. P. (2022). Pesticides removal from aqueous streams through anaerobic and aerobic biological treatment processes. In *Pesticides in the Natural Environment* (pp. 383-418). Elsevier.

Ebrecht, A. C., Smit, M. S., & Opperman, D. J. (2023). Natural alternative heme-environments allow efficient peroxygenase activity by cytochrome P450 monooxygenases. *Catalysis Science & Technology*, *13*(21), 6264-6273.

Ebsa, G., Alemu, T., & Gizaw, B. (2021). Microbial Remediation of Dichloro-Diphenyl Trichloroethane (DDT). *Current Trend in Biotechnology and Microbiology*, *2*(2).

Ebsa, G., Gizaw, B., Admassie, M., Degu, T., & Alemu, T. (2024). The role and mechanisms of microbes in dichlorodiphenyltrichloroethane (DDT) and its residues bioremediation. *Biotechnology Reports*, e00835.

Ekeoma, B. C., Ekeoma, L. N., Yusuf, M., Haruna, A., Ikeogu, C. K., Merican, Z. M. A., & Chelliapan, S. (2023). Recent advances in the biocatalytic mitigation of emerging pollutants: a comprehensive review. *Journal of biotechnology*.

El-Mabrok, M. (2022). Investigating DDT presence in vegetables, fish and human breast milk using analytical chemistry techniques in the Green Mountain area of East Libya.

Encarnação, T., Pais, A.A., Campos, M.G. and Burrows, H.D. (2019). Endocrine disrupting chemicals: Impact on human health, wildlife and the environment. *Science progress*, *102*(1), pp.3-42.

Espinosa-Ortiz, E. J., Rene, E. R., & Gerlach, R. (2022). Potential use of fungal-bacterial co-cultures for the removal of organic pollutants. *Critical reviews in biotechnology*, *42*(3), 361-383.

Fenner-Cris*pp*, P. A. (2010). Risk assessment and risk management: the regulatory process. In *Hayes' Handbook of Pesticide Toxicology* (pp. 1371-1380). Academic Press.

Fester T, Giebler J, Wick LY, *et al*. Plant-microbe interactions as drivers of ecosystem functions relevant for the biodegradation of organic contaminants. Curr Opin Biotechnol. 2014;27:168–175.

Foght, J., April, T., Biggar, K., & Aislabie, J. (2001). Bioremediation of DDT-contaminated soils: a review. *Biorremediación Journal*, *5*(3), 225-246.

Forbes, P., Naudé, Y., & Strumpher, J. (2021). Ongoing Use and Monitoring of DDT in South Africa. *Persistent organic pollutants in the environment: origin and role*, 378.

Gałązka, A., Jankiewicz, U., & Szczepkowski, A. (2023). Biochemical characteristics of laccases and their practical application in the removal of xenobiotics from water. *Applied Sciences*, *13*(7), 4394.

Gao, D., Du, L., Yang, J., Wu, W. M., & Liang, H. (2010). A critical review of the application of white rot fungus to environmental pollution control. *Critical reviews in biotechnology*, *30*(1), 70-77.

Ghosh, S., & Sarkar, B. (2023). Microbial enzymes for biodegradation and detoxification of pesticides. In *Current Developments in Biotechnology and Bioengineering* (pp. 321-356). Elsevier.

Gianfreda, L., Rao, M.A., Scelza, R. and De la Luz Mora, M. (2016). Role of enzymes in environment cleanup/remediation. In *Agro-industrial wastes as feedstock for enzyme production* (pp. 133-155). Academic Press.

Giroux, M. S., Siddiqui, S., & Brander, S. M. (2024). Classic Contaminants in Aquatic Ecosystems: POPs, PFAS, Heavy Metals, and Microplastics. In *Aquatic Ecotoxicology: Understanding Pollutants, Aquatic Organisms, and their Environments* (pp. 43-58). Cham: Springer International Publishing.

Gizaw, B., Alemu, T., Ebsa, G., & Wako, D. (2023). Isolation of Hexavalent chromium tolerant fungal *spp*ecies from urban vegetable farm soil and effluent waste in Addis Ababa& Rift valley, Ethiopia. *World Journal of Microbiology and Biotechnology*, *39*(3), 81.

Gohil, H., & Ogram, A. (2020). The microbial degradation of DDT and potential remediation strategies. *Bioremediation Technology*, 1-24.

Gomathi, S., Ambikapathy, V., & Panneerselvam, A. (2023). Biodegradation and bioaugmentation of pesticides using potential fungal *spp*ecies. In *Plant-Microbe Interaction-Recent Advances in Molecular and Biochemical Approaches* (pp. 79-94). Academic Press.

Gomes, H. I., Dias-Ferreira, C., & Ribeiro, A. B. (2013). Overview of in situ and *Ex situ* remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. *Science of the Total Environment*, *445*, 237-260.

Gonçalves, C. R., & da Silva Delabona, P. (2022). Strategies for bioremediation of pesticides: challenges and perspectives of the Brazilian scenario for global application–a review. *Environmental Advances*, *8*, 100220.

Govarthanan, M., Manikandan, S., Subbaiya, R., Krishnan, R.Y., Srinivasan, S., Karmegam, N., Kim, W. (2022). Emerging trends and nanotechnology advances for sustainable biogas production from lignocellulosic waste biomass: a critical review. *Fuel*, *312*, p.122928.

Guauque-Torres, M. P., & Bustos, A. Y. (2019). Laccases for Soil Bioremediation. *Microbes and Enzymes in Soil Health and Bioremediation*, 165-209.

Gumovskaya, Y. P., Gumovskiy, A. N., Mironova, E. K., Polevshchikov, A. V., Senotrusova, S. V., Usov, V. V., & Tsygankov, V. (2023). Persistent Organic Pollutants in the Human Body: The Experience of Russia and the Former Soviet Republics. In *Persistent Organic Pollutants in the Ecosystems of the North Pacific* (pp. 301-322). Cham: Springer Nature Switzerland.

Gupta, A., Singh, U. B., Sahu, P. K., Paul, S., Kumar, A., Malviya, D., & Saxena, A. K. (2022). Linking soil microbial diversity to modern agriculture practices: a review. *International Journal of Environmental Research and Public Health*, *19*(5), 3141.

Gupta, V.G., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I., Tuohy, M. eds. (2014). *Biotechnology and biology of Trichoderma*. Newnes. https://elsevier.com/locateprmissions, and selecting and obtaining permission, to use Elsevier material. https://elsevier.com/locateprmissions, and selecting and obtaining permission, to use Elsevier material.

Habtamu, K., Teferra, S., & Mihretu, A. (2023). Exploring the perception of key stakeholders toward khat policy approaches in Ethiopia: a qualitative study. *Harm Reduction Journal*, *20*(1), 115.

Hamidi, F., Baghani, A. N., Kasraee, M., Salari, M., & Mehdinejad, M. H. (2023). Modeling, optimization and efficient use of MMT K10 nanoclay for Pb (II) removal using RSM, ANN and GA. *Scientific Reports*, *13*(1), 8434.

Haripriyan, U., Gopinath, K. P., Arun, J., & Govarthanan, M. (2022). Bioremediation of organic pollutants: A mini review on current and critical strategies for wastewater treatment. *Archives of Microbiology*, *204*(5), 286.

Hashemi, S. M., & Damalas, C. A. (2010). Farmers' perceptions of pesticide efficacy: reflections on the importance of pest management practices adoption. *Journal of Sustainable Agriculture*, *35*(1), 69-85.

Hassaan, M. A., Eldeeb, T., & El Nemr, A. (2022). Pesticides removal techniques from the aquatic environment. In *Pesticides in the Natural Environment* (pp. 483-516). Elsevier.

Hleba, L., Hlebova, M., Kovacik, A., Petrova, J., Maskova, Z., Cubon, J., Massanyi, P. (2022). Use of MALDI-TOF MS to Discriminate between Aflatoxin B1-Producing and Non-Producing Strains of A*spp*ergillus flavus. *Molecules*, *27*(22), p.7861.

Huang, D., Gao, L., Zhu, S., Qiao, L., Liu, Y., Ai, Q., Xu, C., Wang, W., Lu, M., Zheng, M. (2023). Target and non-target analysis of organochlorine pesticides and their transformation products in an agrochemical-contaminated area. *Chemosphere*, *324*, p.138314.

Huff, J. (1993). Chemicals and cancer in humans: first evidence in experimental animals. *Environmental health perspectives*, *100*, 201-210.

Hussain, A., Rehman, F., Rafeeq, H., Waqas, M., Asghar, A., Afsheen, N., & Iqbal, H. M. (2022). In-situ, Ex-situ, and nano-remediation strategies to treat polluted soil, water, and air–A review. *Chemosphere*, *289*, 133252.

Ijoma, G.N., Tekere, M. (2017). Potential microbial applications of co-cultures involving ligninolytic fungi in the bioremediation of recalcitrant xenobiotic compounds. *International Journal of Environmental Science and Technology*, *14*, pp.1787-1806.

Islam, M. A., Amin, S. N., Rahman, M. A., Juraimi, A. S., Uddin, M. K., Brown, C. L., & Arshad, A. (2022). Chronic effects of organic pesticides on the aquatic environment and human health: A review. *Environmental Nanotechnology, Monitoring & Management*, *18*, 100740.

Isia, I., Hadibarata, T., Sari, A. A., Al Farraj, D. A., Elshikh, M. S., & Al Khulaifi, M. M. (2019). Potential use of a pathogenic yeast Pichia kluyveri FM012 for degradation of dichlorodiphenyltrichloroethane (DDT). *Water, Air, & Soil Pollution*, *230*, 1-11.

Izcapa-Treviño, C., Loera, O., Tomasini-Campocosio, A., E*spp*arza-García, F., Salazar-Montoya, J. A., Díaz-Cervantes, M. D., & Rodríguez-Vázquez, R. (2009). Fenton (H2O2/Fe) reaction involved in Penicillium *spp*. culture for DDT [1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane)] degradation. *Journal of Environmental Science and Health Part B*, *44*(8), 798-804.

Jansson, J.K., McClure, R. and Egbert, R.G. (2023). Soil microbiome engineering for sustainability in a changing environment. *Nature Biotechnology*, *41*(12), pp.1716-1728.

Jasu, A., Lahiri, D., Nag, M., & Ray, R. R. (2021). Fungi in bioremediation of soil organic pollutants. In *Fungi bio-prospects in sustainable agriculture, environment and nano-technology* (pp. 381-405). Academic Press.

Jones, K. C. (2021). Persistent organic pollutants (POPs) and related chemicals in the global environment: some personal reflections. *Environmental Science & Technology*, *55*(14), 9400-9412.

Jyoti, Yadav, N., Thakur, I. S., & Srivastava, S. (2023). Omics Approach in Nano-Bioremediation of Persistent Organic Pollutants. *Genomics Approach to Bioremediation: Principles, Tools, and Emerging Tec*

Kamalesh, T., Kumar, P. S., & Rangasamy, G. (2023). An insights of organochlorine pesticides categories, properties, eco-toxicity and new developments in bioremediation process. *Environmental Pollution*, 122114.

Karigar, C. S., & Rao, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. *Enzyme research*, *2011*.

Kassegne, A. B., Okonkwo, J. O., Birhanu, T., Daso, A. P., Olukunle, O. I., & Asfaw, S. L. (2020). Ecological risk assessment of organochlorine pesticides and polychlorinated biphenyls in water and surface sediment samples from Akaki River catchment, central Ethiopia. *Emerging Contaminants*, *6*, 396-404.

Khalid, S. A., & Elsherif, W. M. (2022). Types of microorganisms for biodegradation. In *Handbook of biodegradable materials* (pp. 1-27). Cham: Springer International Publishing.

Khaliq, N. (2023). Microbial enzymes as a robust process to mitigate pollutants of environmental concern. In *Microbial Biomolecules* (pp. 241-267). Academic Press.

Khoo, Y. S., Goh, P. S., Lau, W. J., Ismail, A. F., Abdullah, M. S., Ghazali, N. H. M., & salmi Abdullah, N. (2022). Removal of emerging organic micropollutants via modified-reverse osmosis/nanofiltration membranes: A review. *Chemosphere*, *305*, 135151.

Kibria, G., Nugegoda, D., Rose, G., & Haroon, A. Y. (2021). Climate change impacts on pollutants mobilization and interactive effects of climate change and pollutants on toxicity and bioaccumulation of pollutants in estuarine and marine biota and linkage to seafood security. *Marine Pollution Bulletin*, *167*, 112364.

Kolan, A.S., Hall, J.M. (2023). Association of Preterm Birth and Exposure to Endocrine Disrupting Chemicals. International Journal of Molecular Sciences. Jan;24(3):1952.

Kour, D., Kaur, T., Devi, R., Yadav, A., Singh, M., Joshi, D., & Saxena, A. K. (2021). Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. *Environmental Science and Pollution Research*, *28*, 24917-24939.

Krishnani, K.K., Boddu, V.M., Singh, R.D., Chakraborty, P., Verma, A.K., Brooks, L. and Pathak, H. (2023). Plants, animals, and fisheries waste mediated bioremediation of contaminants of environmental and emerging concern (CEECs)–A circular bioresource utilization approach.

Krithiga, T., Sathish, S., Renita, A. A., Prabu, D., Lokesh, S., Geetha, R., & Sillanpaa, M. (2022). Persistent organic pollutants in water resources: Fate, occurrence, characterization and risk analysis. *Science of The Total Environment*, *831*, 154808.

Kumar, A., Nighojkar, A., Varma, P., Prakash, N. J., Kandasubramanian, B., Zimmermann, K., & Dixit, F. (2023). Algal mediated intervention for the retrieval of emerging pollutants from aqueous media. *Journal of Hazardous Materials*, 131568.

Kumar, N., & Shukla, P. (2023). Microalgal-based bioremediation of emerging contaminants: Mechanisms and challenges. *Environmental Pollution*, 122591.

Kumari, S., & Das, S. (2023). Bacterial enzymatic degradation of recalcitrant organic pollutants: Catabolic pathways and genetic regulations. *Environmental Science and Pollution Research*, *30*(33), 79676-79705.

Kulshrestha, G., & Kumari, A. (2010). Simultaneous degradation of mixed insecticides by mixed fungal culture isolated from sewage sludge. *Journal of agricultural and food chemistry*, *58*(22), 11852-11856.

Kuppusamy, S., Palanisami, T., Megharaj, M., Venkateswarlu, K., & Naidu, R. (2016). Ex-situ remediation technologies for environmental pollutants: a critical perspective. *Reviews of Environmental Contamination and Toxicology Volume 236*, 117-192.

Lepke, T. (2003). German Policies on Soil Protection and Remediation of Contaminated Sites. In *Proceedings of the Korean Society of Soil and Groundwater Environment Conference* (pp. 28-57). Korean Society of Soil and Groundwater Environment.

Leskovac, A., Petrović, S. (2023). Pesticide Use and Degradation Strategies: Food Safety, Challenges and Pers*pec*tives. *Foods*, *12*(14), p.2709.

Li, L., Liu, Y., Zhang, Y., Wang, X., Liu, H., Liu, Q., & Zhou, G. (2024). Dynamic responses in Pseudomonas stutzeri M3 bioaugmentation of crude-oil-contaminated soil: Hydrocarbons, microbial community structures, and functional genes. *Journal of Environmental Chemical Engineering*, *12*(1), 111863.

Liu, S., Fang, S., Xiang, Z., Chen, X., Song, Y., Chen, C., & Ouyang, G. (2021). Combined effect of microplastics and DDT on microbial growth: A bacteriological and metabolomics investigation in Escherichia coli. *Journal of Hazardous Materials*, *407*, 124849.

Liu, L., Bai, L., Man, C., Liang, W., Li, F., & Meng, X. (2015). DDT vertical migration and formation of accumulation layer in pesticide-producing sites. *Environmental science & technology*, *49*(15), 9084-9091.

Loha, K. M., Lamoree, M., Weiss, J. M., & de Boer, J. (2018). Import, disposal, and health impacts of pesticides in the East Africa Rift (EAR) zone: A review on management and policy analysis. *Crop protection*, *112*, 322-331.

Ma, J., Pan, L. B., Yang, X. Y., Liu, X. L., Tao, S. Y., Zhao, L., ... & Zhou, Y. Z. (2016). DDT, DDD, and DDE in soil of Xiangfen County, China: residues, sources, spatial distribution, and health risks. *Chemosphere*, *163*, 578-583.

Madbouly, A. K., Rashad, Y. M., Ibrahim, M. I., & Elazab, N. T. (2023). Biodegradation of aflatoxin B1 in maize grains and suppression of its biosynthesis-related genes using endophytic Trichoderma harzianum AYM3. *Journal of Fungi*, *9*(2), 209.

Mahesh, N., Shyamalagowri, S., Pavithra, M. K. S., Alodhayb, A., Alarifi, N., Aravind, J., & Balakumar, S. (2023). Viable remediation techniques to cleansing wastewaters comprising endocrine-disrupting compounds. *Environmental Research*, 116245.

Maja, L. L. (2022). *Assessment of knowledge, attitudes, and safety practices on handling and using pesticides among crop farm pesticides applicators in the Limpopo Province* (Doctoral dissertation, University of Johannesburg).

Malla, M. A., Dubey, A., Kumar, A., Yadav, S., & Kumari, S. (2023). Modeling and optimization of chlorpyrifos and glyphosate biodegradation using RSM and ANN: Elucidating their degradation pathways by GC-MS based metabolomics. *Ecotoxicology and Environmental Safety*, *252*, 114628. 2023.114628.

Mandal, A., Senthil Kumar, P., Poorva, C. S., Srinivasa Raju, L., Balasubramani, S. R., & Rangasamy, G. (2024). Research progress of persistent organic pollutants in water: classification, sources, potential risks, and treatment approaches. *Water Practice & Technology*, wpt2024031.

Mani, V. M., Soundari, A. P. G., Salin, K. P., Mohankumar, R., Preethi, K., Al Obaid, S., & Shanmugam, S. (2023). Optimization parameters for the production of dimer of epicatechin from an endophytic fungus Curvularia australiensis FC2AP using response surface methodology (RSM). *Environmental Research*, *231*, 115962.

Matthiessen, P., Wheeler, J. R., & Weltje, L. (2018). A review of the evidence for endocrine disrupting effects of current-use chemicals on wildlife populations. *Critical reviews in toxicology*, *48*(3), 195-216.

Mawang, C.I., Azman, A.S., Fuad, A.S.M. and Ahamad, M. (2021). Actinobacteria: An eco-friendly and promising technology for the bioaugmentation of contaminants. *Biotechnology Reports*, *32*, p.e00679.

Meftaul, I. M., Venkateswarlu, K., Annamalai, P., Parven, A., & Megharaj, M. (2023). Degradation of four pesticides in five urban landscape soils: human and environmental health risk assessment. *Environmental Geochemistry and Health*, *45*(5), 1599-1614.

Mehta, R. V., Sreenivasa, M. A., Mathew, M., Girard, A. W., Taneja, S., Ranjan, S., & Young, M. F. (2020). A mixed-methods study of pesticide exposures in Breastmilk and Community & Lactating Women's perspectives from Haryana, India. *BMC Public Health*, *20*, 1-14.

Mekonen, S., Ambelu, A., Wondafrash, M., Kolsteren, P., & *Spp*anoghe, P. (2021). Exposure of infants to organochlorine pesticides from breast milk consumption in southwestern Ethiopia. *Scientific reports*, *11*(1), 22053.

Mekonen, S., Lachat, C., Ambelu, A., Steurbaut, W., Kolsteren, P., Jacxsens, L., & *Spp*anoghe, P. (2015). Risk of DDT residue in maize consumed by infants as complementary diet in southwest Ethiopia. *Science of the Total Environment*, *511*, 454-460.

Melendez-Pastor, I., Lopez-Granado, O. M., Navarro-Pedreño, J., Hernández, E. I., Jordán Vidal, M. M., & Gómez Lucas, I. (2023). Environmental factors influencing DDT–DDE spatial distribution in an agricultural drainage system determined by using machine learning techniques. *Environmental Geochemistry and Health*, *45*(12), 9067-9085.

Mengistie, B. T. (2016). Policy-practice nexus: pesticide registration, distribution and use in Ethiopia. *SM J. Environ. Toxicol*, *2*(1), 1006-1019.

Mérida-Ortega, Á, Rothenberg, S. J., Torres-Sánchez, L., Schnaas, L., Hernández-Alcaraz, C., Cebrián, M. E., & López-Carrillo, L. (2019). Polyunsaturated fatty acids and child neurodevelopment among a population exposed to DDT: a cohort study. *Environmental Health*, *18*, 1-7.

Mertens, B. (2006). *Microbial monitoring and degradation of lindane in soil*. Ghent University.

Metcalf, R. L. (1980). Changing role of insecticides in crop protection. *Annual review of Entomology*, *25*(1), 219-256.

Miao, S., Wei, Y., Pan, Y., Wang, Y., & Wei, X. (2023). Detection methods, migration patterns, and health effects of pesticide residues in tea. *Comprehensive Reviews in Food Science and Food Safety*, *22*(4), 2945-2976.

Miglani, R., Parveen, N., Kumar, A., Ansari, M. A., Khanna, S., Rawat, G., & Ansari, M. N. (2022). Degradation of xenobiotic pollutants: an environmentally sustainable approach. *Metabolites*, *12*(9), 818.

Mohapatra, P. (2021). A Critical Review of Effectiveness of the Regulations in India on Designated Persistent Organic Pollutants (POPs) in the Stockholm Convention. *Persistent Organic Pollutants*, 181-200.

Molla, A.H., Fakhru'l-Razi, A., Abd-Aziz, S., Hanafi, M.M. and Alam, M.Z., 2001. In-vitro compatibility evaluation of fungal mixed culture for bioconversion of domestic wastewater sludge. *World Journal of Microbiology and Biotechnology*, *17*, pp.849-856.

Mori, T., Sudo, S., Kawagishi, H. and Hirai, H. (2018). Biodegradation of diuron in artificially contaminated water and seawater by wood colonized with the white-rot fungus Trametes versicolor. *Journal of Wood Science*, *64*(5), pp.690-696.

Mrema, E.J., Rubino, F.M., Colosio, C. (2013). Obsolete pesticides: a threat to environment, biodiversity, and human health, p 1–21. *In* Simeonov LI, Macaev FZ, Simeonova BG (ed), *Environmental security assessment and management of obsolete pesticides in Southeast Europe*. NATO science for peace and security series C: environmental security. Springer, Dordrecht, Netherlands.

Mukasa, P., Wasswa, J., Namuyomba, P., & Ntambi, E. (2022). Dichlorodiphenyltrichloroethane (DDT) and its metabolites in house dust, soil and selected food crops from indoor residual sprayed areas of Apac and Oyam Districts, Uganda. *African Journal of Pure and Applied Chemistry*, *16*(2), 28-39.

Muter, O. (2023). Current trends in bioaugmentation tools for bioremediation: A critical review of advances and knowledge gaps. *Microorganisms*, *11*(3), 710.f

Nadeau, L. J., Menn, F. M., Breen, A., & Sayler, G. S. (1994). Aerobic degradation of 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) by Alcaligenes eutrophus A5. *Applied and environmental microbiology*, *60*(1), 51-55.

Nagar, N., Saxena, H., Pathak, A., Mishra, A., & Poluri, K. M. (2023). A review on structural mechanisms of protein-persistent organic pollutant (POP) interactions. *Chemosphere*, 138877.

Naik, S. R., Gamare, D., & Bhopatrao, A. (2024). Chemical health hazards and toxicity of environmental pollutants on humans, animals and others: An overview. *Journal of Toxicological Studies*, *2*(1), 1135-1135.

Obotey Ezugbe, E., & Rathilal, S. (2020). Membrane technologies in wastewater treatment: a review. *Membranes*, *10*(5), 89.

Okçu, G. D., Pakdil, N. B., Ökten, H. E., & Yalçuk, A. (2018). A Box-Behnken design (BBD) optimization of the photocatalytic degradation of 2, 4-dichlorophenoxyacetic acid (2, 4-D) using TiO2/H2O. *Desalination and Water Treatment*.

Pande, V., Pandey, S. C., Sati, D., Pande, V., & Samant, M. (2020). Bioremediation: an emerging effective approach towards environment restoration. *Environmental Sustainability*, *3*, 91-103.

Pandey, A., Kumar, S., Kumar, S., Rajput, M., Singh, M., Pandey, C., & Bhardwaj, N. (2023). Role of microbial enzymes in bioremediation: Emerging opportunities and limitations. In *Microbial Inoculants* (pp. 277-300). Academic Press.

Pandey, A., Singh, P., Gupta, D., Fatima, A. Prasad, S., M. (2021). Utilization of Advanced Technologies for in-situ Remediation of Polluted Soil to avoid Ecological Risks: A Review.

Pang, W., Gao, N., & Xia, S. (2010). Removal of DDT in drinking water using nanofiltration process. *Desalination*, *250*(2), 553-556.

Panwar, R., Mathur, J. (2023). Remediation of polycyclic aromatic hydrocarbon-contaminated soils using microbes and nanoparticles: A review. *Pedosphere*, *33*(1), pp.93-104.

Parial, D., & Dey, S. (2023). Role of Microalgae in Wastewater Treatment and Their Role in Nutrient Recovery. In *Sustainable Industrial Wastewater Treatment and Pollution Control* (pp. 31-53). Singapore: Springer Nature Singapore.

Parra-Arroyo, L., González-González, R. B., Castillo-Zacarías, C., Martínez, E. M. M., Sosa-Hernández, J. E., Bilal, M., ... & Parra-Saldívar, R. (2022). Highly hazardous pesticides and related pollutants: Toxicological, regulatory, and analytical aspects. *Science of The Total Environment*, *807*, 151879.

Paul, C., Pal, N., Maitra, M., & Das, N. (2024). Laccase-assisted Bioremediation of Pesticides: Scope and Challenges. *Mini-Reviews in Organic Chemistry*, *21*(6), 633-654.

Paul, D., & Mandal, S. M. (2019). Microbial adaptation and resistance to pesticides. *Bacterial Adaptation to Co-Resistance*, 233-249.

Persiani, A.M., Maggi, O., Montalvo, J., Casado, M.A. and Pineda, F.D. (2008). Mediterranean grassland soil fungi: patterns of biodiversity, functional redundancy and soil carbon storage *Plant Biosystems*, *142*(1), pp.111-119.

Porwal, S., Chaudhary, S., Singh, A., Varma, A., 2023. Microbial Products: Applications in the Field of Biotechnology and Bioremediation. InMicrobial Products (pp. 3-10). CRC Press.

Pradhan, B., Chand, S., Chand, S., Rout, P. R., & Naik, S. K. (2023). Emerging groundwater contaminants: A comprehensive review on their health hazards and remediation technologies. *Groundwater for Sustainable Development*, *20*, 100868.

Pradhan, S. S., Gowda, G. B., Adak, T., Guru-Pirasanna-Pandi, G., Patil, N. B., Annamalai, M., & Rath, P. C. (2022). Pesticides occurrence in water sources and decontamination techniques. In *Pesticides-Updates on Toxicity, Efficacy and Risk Assessment*. IntechOpen.

Pryce, J., Medley, N., & Choi, L. (2022). Indoor residual spraying for preventing malaria in communities using insecticide-treated nets. *Cochrane Database of Systematic Reviews*, (1).

Purnomo, A.S., Sariwati, A., Fatmawati, S., Pus*pp*itasari, F.E. (2023). Effect of the Coconut Coir (Cocos nucifera) as a Growth Medium for Pleurotus ostreatus (Oyster Mushroom) on Mineral and Vitamin B Contents. *HAYATI Journal of Biosciences*, *30*(1), pp.95-104.

Purnomo, A. S., Sariwati, A., & Kamei, I. (2020). Synergistic interaction of a consortium of the brown-rot fungus Fomitopsis pinicola and the bacterium Ralstonia pickettii for DDT biodegradation. *Heliyon*, *6*(6).

Purnomo, A.S., Putra, S.R., Shimizu, K., Kondo, R. (2014). Biodegradation of heptachlor and heptachlor epoxide-contaminated soils by white-rot fungal inocula. *Environmental Science and Pollution Research*, *21*, pp.11305-11312.

Purnomo, A. S., Kamei, I., & Kondo, R. (2008). Degradation of 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) by brown-rot fungi. *Journal of bioscience and bioengineering*, *105*(6), 614-621.

Purnomo, A. S., Ashari, K., & Hermansyah, F. T. (2017). Evaluation of the synergistic effect of mixed cultures of white-rot fungus Pleurotus ostreatus and biosurfactant-producing bacteria on DDT biodegradation. *Journal of Microbiology and Biotechnology*, *27*(7), 1306-1315.

Qi, S. Y., Xu, X. L., Ma, W. Z., Deng, S. L., Lian, Z. X., & Yu, K. (2022). Effects of organochlorine pesticide residues in maternal body on infants. *Frontiers in Endocrinology*, *13*, 890307.

Qin, H., Zha, T., Qian, K., Sun, Y., Guan, X., & Chen, C. (2023). Efficient full dechlorination of chlorinated ethenes on single enzyme-like Co− N4 sites in nitrogen-doped carbons. *Applied Catalysis B: Environmental*, *328*, 122459.

Rabbani, A., Mishra, A. K., Goyal, N., & Arora, S. (2024). Green Technologies for Pesticide Contaminated Soil and Water. *Sustainable Production Innovations: Bioremediation and Other Biotechnologies*, 163-192.

Rachel, C. (1962). *Silent* s*pring*. Penguin Books.

Rafeeq, H., Afsheen, N., Rafique, S., Arshad, A., Intisar, M., Hussain, A., & Iqbal, H. M. (2023). Genetically engineered microorganisms for environmental remediation. *Chemosphere*, *310*, 136751.

Raffa, C. M., & Chiampo, F. (2021). Bioremediation of agricultural soils polluted with pesticides: A review. *Bioengineering*, *8*(7), 92.

Rajak, P., Roy, S., Ganguly, A., Mandi, M., Dutta, A., Das, K., & Biswas, G. (2023). Agricultural pesticides–friends or foes to biosphere? *Journal of Hazardous Materials Advances*, *10*, 100264.

Rajput, S., Sharma, R., Kumari, A., Kaur, R., Sharma, G., Arora, S. and Kaur, R. (2022). Pesticide residues in various environmental and biological matrices: Distribution, extraction, and analytical procedures. *Environment, Development and Sustainability*, *24*(5), pp.6032-6052.

Rani, A., Rana, A., Dhaka, R. K., Singh, A. P., Chahar, M., Singh, S., & Minz, D. (2023). Bacterial volatile organic compounds as biopesticides, growth promoters and plant-defense elicitors: Current understanding and future scope. *Biotechnology Advances*, *63*, 108078.

Rao, A., & Morimoto, R. (2020). *An Analysis of the use of Chemical Pesticides and their Impact on Yields, Farmer Income and Agricultural Sustainability: The Case for Smallholder Farmers in Ethiopia* (No. 234).

Rashmi, I., Karthika, K. S., Roy, T., Shinoji, K. C., Kumawat, A., Kala, S., & Pal, R. (2022). Soil Erosion and sediments: A source of contamination and impact on agriculture productivity. In *Agrochemicals in Soil and Environment: Impacts and Remediation* (pp. 313-345). Singapore: Springer Nature Singapore.

Rasmussen, K., Rauscher, H., Kearns, P., González, M. and Sintes, J.R. (2019). Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data. *Regulatory Toxicology and Pharmacology*, *104*, pp.74-83.

Ray, S. S., Parihar, K., Goyal, N., & Mahapatra, D. M. (2024). Synergistic Insights into Pesticide Persistence and Microbial Dynamics for Bioremediation. *Environmental Research*, 119290.

Reddy, K., Jose, S., Fayaz, T., Renuka, N., Ratha, S.K., Kumari, S. and Bux, F. (2024). Microbe-Assisted Bioremediation of Pesticides from Contaminated Habitats. *Bioremediation for Sustainable Environmental Cleanup*, p.109.

Rehman, M. U., Wali, A. F., & Bajaj, V. (Eds.). (2024). *Pesticide Toxicology and Risk Assessment: Linking Exposure to Biological Effects*. CRC Press.

Rezende-Teixeira, P., Dusi, R. G., Jimenez, P. C., E*spp*indola, L. S., & Costa-Lotufo, L. V. (2022). What can we learn from commercial insecticides? Efficacy, toxicity, environmental impacts, and future developments. *Environmental Pollution*, *300*, 118983.

Riyaz, M., Mathew, P., Shah, R.A., Sivasankaran, K., Zuber, SM. (2023). Environmental Pesticide Degradation: Mechanisms and Sustainability. InBioremediation and Phytoremediation Technologies in Sustainable Soil Management (pp. 3-51). Apple Academic Press.

Rizqi, H. D., Purnomo, A. S., & Ulfi, A. (2023). The effect of bacteria addition on DDT biodegradation by BROWN-ROT fungus Gloeophyllum trabeum. *Heliyon*, *9*(7).

Robert, B., Chenthamara, D., & Subramaniam, S. (2022). Fabrication and biomedical applications of Arabinoxylan, Pectin, Chitosan, soy protein, and silk fibroin hydrogels via laccase-Ferulic acid redox chemistry. *International Journal of Biological Macromolecules*, *201*, 539-556.

Rose, M. (2023). Pollutants, residues and other contaminants in foods obtained from marine and fresh water. In *Present knowledge in food safety* (pp. 128-141). Academic Press.

Rusiecki, J. A., McAdam, J., Denic-Roberts, H., Sjodin, A., Davis, M., Jones, R., & Zhang, Y. (2024). Organochlorine pesticides and risk of papillary thyroid cancer in US military personnel: a nested case-control study. *Environmental Health*, *23*(1), 28.

Russo, F., Ceci, A., Pinzari, F., Siciliano, A., Guida, M., Malusà, E., Tartanus, M., Miszczak, A., Maggi, O., Persiani, A.M. (2019). Bioremediation of dichlorodiphenyltrichloroethane (DDT)-contaminated agricultural soils: potential of two autochthonous saprotrophic fungal strains. *Applied and environmental microbiology*, *85*(21), pp.e01720-19.

Sadasivaiah, S., Tozan, Y., & Breman, J. G. (2007). Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: how can it be used for malaria control?. *Defining and Defeating the Intolerable Burden of Malaria III: Progress and* Perspectives*: Supplement to Volume 77 (6) of American Journal of Tropical Medicine and Hygiene*.

Salem, T. M., Fetyan, N. A., Taha, A. A., Malhat, F. M., & Ramadan, A. I. (2023). Covalent Immobilization of Multi-Enzyme Biocatalysts on Nano-Silica for Efficient p, p'-DDT Degradation.

Sana, S., Qadir, A., Mumtaz, M., Evans, N. P., & Ahmad, S. R. (2021). Spatial trends and human health risks of organochlorinated pesticides from bovine milk; a case study from a developing country, Pakistan. *Chemosphere*, *276*, 130110.

Sang, C., Yu, Z., An, W., Sørensen, P. B., Jin, F., & Yang, M. (2022). Development of a data driven model to screen the priority control pesticides in drinking water based on health risk ranking and contribution rates. *Environment International*, *158*, 106901.

Santacruz, G., Bandala, E. R., & Torres, L. G. (2005). Chlorinated pesticides (2, 4-D and DDT) biodegradation at high concentrations using immobilized Pseudomonas fluorescens. *Journal of Environmental Science and Health Part B*, *40*(4), 571-583.

Saravanan, A., Kumar, P. S., Vo, D. V. N., Jeevanantham, S., Karishma, S., & Yaashikaa, P. R. (2021). A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. *Journal of Hazardous Materials*, *419*, 126451.

Sari, A. A., Tachibana, S., & Muryanto. (2012). Correlation of ligninolytic enzymes from the newly-found *spp*ecies Trametes versicolor U97 with RBBR decolorization and DDT degradation. *Water, Air, & Soil Pollution*, *223*, 5781-5792.

Satish, G., Ashokrao, D.M. and Arun, S.K. (2017). Microbial degradation of pesticide: a review. *African journal of microbiology research*, *11*(24), pp.992-1012.

Sawant, C. G., Sharma, L. D., & Sadhukhan, R. (2022). Pesticides, its Residues and Environment. *Pesticide Residues*, 11.

Sehrawat, A., Phour, M., Kumar, R., & Sindhu, S. S. (2021). Bioremediation of pesticides: an eco-friendly approach for environment sustainability. *Microbial Rejuvenation of Polluted Environment: Volume 1*, 23-84.

Sesin, V., Judy, J. D., Kapustka, L., Opeolu, B., Ottinger, M. A., Bertsch, P. M., & Stahl Jr, R. G. (2023). The Importance of Fostering and Funding Scientific Research, and its Relevance to Environmental Toxicology and Chemistry. *Environmental Toxicology and Chemistry*, *42*(3), 581-593.

Shahbaz, A., Hussain, N., Mahmood, T., Ashraf, M., & Khaliq, N. (2023). Environment-Friendly Microbial Bioremediation. *Microbial Bioreactors for Industrial Molecules*, 397-419.

Shahid, M., Khan, M. S., & Singh, U. B. (2023). Pesticide-tolerant microbial consortia: Potential candidates for remediation/clean-up of pesticide-contaminated agricultural soil. *Environmental Research*, 116724.

Sharma, A., Richa, Anu, & Kumari, A. (2022). Microbial degradation of industrial pollutants from different environments. In *Microbial bioremediation: sustainable management of environmental contamination* (pp. 215-238). Cham: Springer International Publishing.

Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., & Singla, N. (2020). Global trends in pesticides: A looming threat and viable alternatives. *Ecotoxicology and Environmental Safety*, *201*, 110812.

Seo, J. S., Keum, Y. S., & Li, Q. X. (2009). Bacterial degradation of aromatic compounds. *International journal of environmental research and public health*, *6*(1), 278-309.

Sheikh, S., Miranda, J., Rai, V., Sheikh, S., & Shameena, K. A. (2023). Influence of subsoil microbial community across different depths on the biodegradation of pesticides. In *Current Developments in Biotechnology and Bioengineering* (pp. 379-400). Elsevier.

Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011). Laccase: microbial sources, production, purification, and potential biotechnological applications. *Enzyme research*, *2011*.

Sherif, H., & Mostafa, A. A. (2014). Mycoremediation of organophosphorous insecticide chlorpyrifos by fungal soil isolates. *Journal of pure and applied microbiology*, *8*(4), 2945-2951.

Shi, L., Shen, C., Zhang, P., Xu, J., Wu, X., Pan, X., & Zheng, Y. (2023). Assessment on the stereoselective behavior of cyflumetofen to earthworms (Eisenia foetida): Degradation, bioaccumulation, toxicity mechanism, and metabolites. *Science of The Total Environment*, 164541.

Silva, A.M., Jones, D.L., Chadwick, D.R., Qi, X., Cotta, S.R., Araújo, V.L., Matteoli, F.P., Lacerda-Júnior, G.V., Pereira, A.P., Fernandes-Júnior, P.I., Cardoso, E.J. (2023). Can arbuscular mycorrhizal fungi and rhizobacteria facilitate 33P uptake in maize plants under water stress? *Microbiological research*, *271*, p.127350.

Singh, V., & Sable, H. (2024). Bioremediation of emerging pollutants: a sustainable remediation approach. In *Emerging Contaminants* (pp. 335-361). Woodhead Publishing.

Singh, A., Kumari, R. and Yadav, A.N. (2021a). Fungal secondary metabolites for bioremediation of hazardous heavy metals. *Recent Trends in Mycological Research: Volume 2: Environmental and Industrial Perspective*, pp.65-98.

Singh, G., Singh, A., Singh, P., Shukla, R., Tripathi, S., & Mishra, V. K. (2021). The fate of organic pollutants and their microbial degradation in water bodies. *Pollutants and water management: resources, strategies and scarcity*, 210-240.

Singh, M., Singh, D., Rai, P.K., Suyal, D.C., Saurabh, S., Soni, R., Giri, K. and Yadav, A.N. (2021b). Fungi in remediation of hazardous wastes: current status and future outlook. *Recent Trends in Mycological Research: Volume 2: Environmental and Industrial Perspective*, pp.195-224.

Smith, A. G. (2024). GABAergic Neurotransmission and Toxicity 1: Organochlorines.

Sousa, S., Maia, M. L., Correira-Sá, L., Fernandes, V. C., Delerue-Matos, C., Calhau, C., & Domingues, V. F. (2020). Chemistry and toxicology behind insecticides and herbicides. *Controlled release of pesticides for sustainable agriculture*, 59-109.

Sullivan, M. M. (2004). *Breaking silence with the sounds of the sea: Other words on Rachel Carson and the three sea books*. Bowling Green State University.

Suman, S., & Tanuja. (2021). Isolation and Characterization of a Bacterial Strain Enterobacter cloacae (Accession No. KX438060. 1) Capable of Degrading DDTs Under Aerobic Conditions and Its Use in Bioremediation of Contaminated Soil. *Microbiology Insights*, *14*, 11786361211024289.

Susila, E., Maulina, F., Emilda, D. (2023). Characterization and identification of Trichoderma on shallots isolated from three elevation regions in West Sumatra, Indonesia. *BIODIVERSITAS*, *24*(4), pp.2064-2071.

Tadele, T., & Amado, Z. (2023). Determination of the level of pesticide residues, heavy metals and physicochemical compositions of cow's milk in Hawassa city, Ethiopia. *International Journal of Research*, *9*(2), 1-17.

Tarfeen, N., Nisa, K. U., Hamid, B., Bashir, Z., Yatoo, A. M., Dar, M. A., & Sayyed, R. Z. (2022). Microbial remediation: A promising tool for reclamation of contaminated sites with *spp*ecial9 emphasis on heavy metal and pesticide pollution: A review. *Processes*, *10*(7), 1358.

Tarla, D. N., Erickson, L. E., Hettiarachchi, G. M., Amadi, S. I., Galkaduwa, M., Davis, L. C., ... & Pidlisnyuk, V. (2020). Phytoremediation and bioremediation of pesticide-contaminated soil. *Applied Sciences*, *10*(4), 1217.

Tasselli, S., Marziali, L., Roscioli, C., Guzzella, L. (2023). Legacy Dichlorodiphenyltrichloroethane (DDT) Pollution in a River Ecosystem: Sediment Contamination and Bioaccumulation in Benthic Invertebrates. *Sustainability*, *15*(8), p.6493.

Tesema, M., Asefa, A., & Delelegn, Y. (2022). ASSESSMENT: RANKING MAJOR THREATS IMPACTING ETHIOPIA'S.

Teshome, A., Terefe, G., Getaw, H., & Koomen, I. (2023). Pesticide Lifecycle in Ethiopia: challenges, opportunities and leverage points.

Thirumoorthy, S.G., Pavithran, S., Lakshmaiam, V.V., Nagella, P., 2023. Toxicity and Genetic Basis of Microbial Degradation of Organophosphorus Pesticides. InBioremediation and

Phytoremediation Technologies in Sustainable Soil Management (pp. 379-426). Apple Academic Press. 9781003281207.

Tissot, A. N., Murphey, M., & Waites, R. E. (1954). A brief history of entomology in Florida. *The Florida Entomologist*, *37*(2), 51-71.

Tonello, C.Z., Correa, N.B., Colla, L.M. (2023). Bioremediation of terbuthylazine contaminated soil: a bibliometric and systematic review. *Conjecturas*, *23*(2), pp.157-177.

Tortella, G.R., Diez, M.C. and Durán, N. (2005). Fungal diversity and use in decomposition of environmental pollutants. *Critical Reviews in Microbiology*, *31*(4), pp.197-212.

Tsuchida, S., Yamashita, K., Murata, S., Miyabe, A., Satoh, M., Matsushita, K., Nakayama, T., Nomura, F. and Umemura, H. (2023). Evaluation of a novel sample preparation method for identifying A*spp*ergillus fumigatus using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Combining Yatalase and silica beads treatment. *Journal of Microbiological Methods*, *207*, p.106706.

Umulisa, V., Kalisa, D., Skutlarek, D., & Reichert, B. (2020). First evaluation of DDT (dichlorodiphenyltrichloroethane) residues and other Persistence Organic Pollutants in soils of Rwanda: Nyabarongo urban versus rural wetlands. *Ecotoxicology and environmental safety*, *197*, 110574.

United States Environmental Protection Agency (USEPA, 1975). "DDT regulatory history: A brief survey? To 1975." USEPA, http://www.epa.gov/history/topics/ddt/02.htm?? July 20, 2005.

Usip, P. U., Ekong, U. G., Mfon, E. N., Panchal, R. K., Osang, F. B., & Asuquo, D. E. (2023). An Intelligent Multilingual Farm Advisory System for Controlling Hazardous Effects of Pesticides on Human Health. In *SWIoT+ MSW@ KGSWC* (pp. 51-74).

Vaksmaa, A., Guerrero-Cruz, S., Ghosh, P., Zeghal, E., Hernando-Morales, V., Niemann, H. (2023). Role of fungi in bioremediation of emerging pollutants. *Frontiers in Marine Science*, *10*, p.1070905.

van Genuchten, E. (2023). Pollution Solutions: Removing Pollutants from Soil and Water. In *A Guide to a Healthier Planet: Scientific Insights and Actionable Steps to Help Resolve Climate, Pollution and Biodiversity Issues* (pp. 83-96). Cham: Springer Nature Switzerland.

Velasco, A., Aburto-Medina, A., Shahsavari, E., Revah, S., & Ortiz, I. (2017). Degradation mechanisms of DDX induced by the addition of toluene and glycerol as cosubstrates in a zero-valent iron pretreated soil. *Journal of hazardous materials*, *321*, 681-689.

Vermeulen, E., Verhaegen, J., Indevuyst, C., & Lagrou, K. (2012). Update on the evolving role of MALDI-TOF MS for laboratory diagnosis of fungal infections. *Current Fungal Infection Reports*, *6*, 206-214.

Vijayanand, M., Ramakrishnan, A., Subramanian, R., Issac, P. K., Nasr, M., Khoo, K., & Ravindran, B. (2023). Polyaromatic hydrocarbons (PAHs) in the water environment: A review on toxicity, microbial biodegradation, systematic biological advancements, and environmental fate. *Environmental research*, 115716.

Wan, M. L. Y., Co, V. A., & El-Nezami, H. (2022). Endocrine disrupting chemicals and breast cancer: a systematic review of epidemiological studies. *Critical Reviews in Food Science and Nutrition*, *62*(24), 6549-6576.

Wang, X., Oba, B. T., Wang, H., Luo, Q., Liu, J., Tang, L., & Sun, L. (2023). Degradation of DDT by a novel bacterium, Arthrobacter globiformis DC-1: efficacy, mechanism and comparative advantage. *Water*, *15*(15), 2723.

Wang, X., Sial, M. U., Bashir, M. A., Bilal, M., Raza, Q. U. A., Ali Raza, H. M., & Geng, Y. (2022). Pesticides xenobiotics in soil ecosystem and their remediation approaches. *Sustainability*, *14*(6), 3353.

Wang, R., Qu, C., Li, M., Shi, C., Li, W., Zhang, J., & Qi, S. (2021). Health risks of exposure to soil-borne dichlorodiphenyltrichloroethanes (DDTs): A preliminary probabilistic assessment and spatial visualization. *Science of the Total Environment*, *772*, 144949.

Wang, G., Zhang, J., Wang, L., Liang, B., Chen, K., Li, S., & Jiang, J. (2010). Co-metabolism of DDT by the newly isolated bacterium, Pseudoxanthomonas *spp*. wax. *Brazilian Journal of Microbiology*, *41*, 431-438.

Watanabe, T., 2010. Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to *Spp*ecies. 3rd. Ed. – CRC Press, Florida. 9781439804193.

Wilkes, T.I., Warner, D.J., Edmonds-Brown, V., Davies, K.G., Denholm, I. (2021). The tripartite Rhizobacteria-AM fungal-host plant relationship in winter wheat: Impact of multi-*spp*ecies inoculation, tillage regime and naturally occurring Rhizobacteria *spp*ecies. *Plants*, *10*(7), p.1357.

Wm-Bekele, D., Girma Tilahun, Dadebo, E., Haileslassie, A., & Gebremariam, Z. (2024). Organochlorine, organopho*sph*orus, and carbamate pesticide residues in an Ethiopian Rift

Valley Lake Hawassa: occurrences and possible ecological risks. *Environmental Science and Pollution Research*, *31*(19), 27749-27769.

Wolfe, N. L., Zepp, R. G., Paris, D. F., Baughman, G. L., & Hollis, R. C. (1977). Methoxychlor and DDT degradation in water: rates and products. *Environmental Science & Technology*, *11*(12), 1077-1081.

World Health Organization. (1990). *Public health impact of pesticides used in agriculture*. World Health Organization.

World Health Organization. (1979). *Environmental Health Criteria 9. DDT and its derivatives*. United Nations Environment Programme/WHO, Geneva, Switzerland.

Wu, D., Wang, W., Yao, Y., Li, H., Wang, Q., & Niu, B. (2023). Microbial interactions within beneficial consortia promote soil health. *Science of The Total Environment*, 165801.

Xiao, Y., Wu, C., Cui, P., Zhou, L., Yin, Q. (2023). Pursuing Green and Efficient Agriculture from Molecular Assembly: A Review of Solid-State Forms on Agrochemicals. *Journal of Agricultural and Food Chemistry*.

Xie, H., Zhu, L., Xu, Q., Wang, J., Liu, W., Jiang, J., & Meng, Y. (2011). Isolation and degradation ability of the DDT-degrading bacterial strain KK. *Environmental Earth Sciences*, *62*, 93-99.

Xu, Y., Yang, X., Chen, D., Xu, Y., Lan, L., Zhao, S., & Xia, Y. (2023). Maternal exposure to pesticides and autism or attention-deficit/hyperactivity disorders in off*spr*ing: A meta-analysis. *Chemosphere*, *313*, 137459.

Yadav, S., & Chandra, R. (2020). Biofilm-mediated bioremediation of pollutants from the environment for sustainable development. In *New and future developments in microbial biotechnology and bioengineering: microbial biofilms* (pp. 177-203). Elsevier.

Yang, M., Park, M.S. and Lee, H.S. (2006). Endocrine disrupting chemicals: human exposure and health risks. *Journal of Environmental Science and Health Part C*, *24*(2), pp.183-224.

Yu, Z., Lu, T., & Qian, H. (2023). Pesticide interference and additional effects on plant microbiomes. *Science of The Total Environment*, *888*, 164149.

Zainith, S., Chowdhary, P., Mani, S., & Mishra, S. (2020). Microbial ligninolytic enzymes and their role in bioremediation. In *Microorganisms for Sustainable Environment and Health* (pp. 179-203). Elsevier.

Zheng, Q., Xiao, J., Zhang, D., Li, X., Xu, J., Ma, J., & Lu, S. (2024). Bi*spp*henol analogues in infant foods in south China and implications for infant exposure. *Science of The Total Environment*, *910*, 168509.

Zhang, H., Yuan, X., Xiong, T., Wang, H., & Jiang, L. (2020). Bioremediation of co-contaminated soil with heavy metals and pesticides: Influence factors, mechanisms and evaluation methods. *Chemical Engineering Journal*, *398*, 125657.

Zulfiqar, U., Haider, F. U., Maqsood, M. F., Mohy-Ud-Din, W., Shabaan, M., Ahmad, M., & Shahzad, B. (2023). Recent advances in microbial-assisted remediation of cadmium-contaminated soil. *Plants*, *12*(17), 3147.

Appendixes
Appendix I Research paper (Published)

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Screening, characterization and optimization for synergistic interaction of potential dichlorodiphenyltrichloroethane degrading fungi isolated from agro-industrial effluent and farm soil

Girma Ebsa [a], Birhanu Gizaw [b], Tesfaye Alemu [a,*]

[a] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P. O. Box: 1176, Addis Ababa, Ethiopia
[b] Ethiopian Biodiversity Institute, Ethiopia

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Keywords:
Dichlorodiphenyltrichloroethane
Degradation
Gas chromatography-electron captured detector
Matrix-assisted laser desorption
Ionization
Optimization

ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is a recalcitrant synthetic chemical that threatens the environment. Despite being banned in most industrialized nations, DDT is still used as a pesticide to fight malaria and agricultural pests. The main objective of this study is to screen, characterize, and evaluate potential DDT-degrading fungi and their synergetic interaction effects for mycoremediation purpose. The soil and effluent samples were collected from Ziway, Koka, and Addis Ababa. Fungal isolation and screening were performed using a serial dilution on potato dextrose agar growth media. Matrix-Assisted Laser Desorption, Ionization, and Time of Flight Mass Spectrometry technology was used for fungal identification and the selected isolates AS1 and T1 were identified as *Aspergillus niger and Trichoderma koningii*. Fungal biomass production and sporulation capacity were examined and optimized using a Box-Behnken experimental design. The potential DDT-tolerant fungi were studied based on growth factor optimization. The optimization results revealed that the co-inoculated isolates AS1T1 had a maximum biomass (1.01 ± 0.16g) and spore count (5.74 ± 0.37 log spore/mL) and were selected as possible DDT-degrading fungi. Gas Chromatograph-Electron Capture Detector technology was used for the DDT degradation study. Its analysis confirmed that fungal-co-cultured *Aspergillus niger* and *Trichoderma koningii* in DDT-amended liquid medium were able to degrade DDT into its metabolites (DDE and DDD). The results also revealed that 99.5–99.99% of DDT and its metabolites degraded from initial concentrations of 1750, 3500, 5250, and 7000 ppm. The co-inoculated fungi *Aspergillus niger* and *Trichoderma koningii* are promising candidates for the removal of DDT and its metabolites from polluted environments.

1. Introduction

Dichlorodiphenyltrichloroethane (DDT) is a persistent organochlorine synthetic chemical used on a global scale to control agricultural pests and the spread of diseases caused by pests (Kumar and Mukherji, 2018; Burgos-Aceves et al., 2021; Kolan and Hall, 2023). Although DDT chemicals increase longevity in using for malaria control and agricultural productivity, concerns have been raised about the environmental dangers cause to both humans and wildlife. The threats to human health from DDT exposure are both

* Corresponding author.
E-mail addresses: girmaebsa4@gmail.com (G. Ebsa), gizachewbirhan@gmail.com (B. Gizaw), tesfayeAfrica12@gmail.com, tesfaye.alemu@aau.edu.et (T. Alemu).

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Appendix II Research paper (Published)

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Degradation of Dichlorodiphenyltrichloroethane (DDT) and its main metabolites (Diphenyldichloroethylene (DDE) and Dichlorodiphenyldichloroethane (DDD) using Trichoderma species

Girma Ebsa [a,*], Birhanu Gizaw [b], Mesele Admassie [a], Tizazu Degu [c], Asnake Desalegn [a], Eshetu Yilma [a], Wondwosen Melak [a], Fiseha Tadesse [a], Tesfaye Alemu [a,*]

[a] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P. O. Box: 1176, Addis Ababa, Ethiopia
[b] Department of Microbial Biodiversity Directorate, Ethiopian Biodiversity Institute, P. O. Box: 1176, Addis Ababa, Ethiopia
[c] Department of Crop Protection, Ethiopian Institute of Agricultural Research, P. O. Box: 2003, Addis Ababa, Ethiopia

ARTICLE INFO

Keywords:
Dichlorodiphenyldichloroethane (DDD)
Diphenyldichloroethylene (DDE)
Dichlorodiphenyltrichloroethane (DDT)
Degradation
GC-ECD
MALDI-TOF
Optimization

ABSTRACT

Trichoderma species' ability to metabolize a variety of pesticides has led to their widespread use in agriculture. Dichlorodiphenyltrichloroethane (DDT) is a recalcitrant xenobiotic compound that endangers both fauna and flora. The aim of this study is to screen, characterize, and evaluate potential Trichoderma species that have the capability to degrade DDT and its main metabolites. Soil and effluent water samples were collected from Addis Ababa, Koka, and Ziway. The enrichment culture technique was used to isolate Trichoderma species Based on its morphological characteristics and MALDI-TOF MS analysis identification, the isolate that was ultimately chosen was determined to be Trichoderma orientale. A general, full factorial design was employed in the optimization assay. The highest biomass (0.09 ± 0.14 g), spore count (5.71 ± 0.55 log/mL), and radial growth rate (6.7 ± 3.1 cm) of isolate T4 was discovered. Using a gas chromatograph-electron capture detector, the degradation assays were evaluated from DDT-amended liquid media. At initial concentrations of 1750, 3500, 5250, and 7000 mg/L, it was able to co-metabolize and degrade more than 96 % of DDT, DDE, and DDD simultaneously. The T. orientale had the highest degradation efficiency among all the reported DDT-degrading Trichoderma species. This study demonstrates the potential use of T. orientale for DDT bioremediation purposes.

1. Introduction

Nearly all of the natural resources that are essential to human survival have become contaminated as a result of industrial expansion [1]. Environmental pollutants are inorganic or organic in nature, miscellaneous in their toxicity to living organisms, persist in the environment, and biomagnified in the food chain [2,3]. The most frequently detected contaminants are pesticides, which are challenging human health globally. These contaminants can cause cancer, allergies, neurological disorders, cardiovascular and kidney diseases, lung fibrosis, and reproductive disorders [2,4,5]. It is estimated that about 4.9 million of the world population dies annually due to industrial contamination. This increasing rate of natural resource contamination needs to be improved immediately with sustainable, low-cost, and eco-friendly technologies [6–8]. Ecological contamination with pesticides is one of the most serious threats to the global community due to their potential toxicity, high persistence, slow degradation, and bioaccumulation [9,10]. DDT is one of the persistent organic pollutants that was widely used in the 1940s as a pesticide to control agricultural crop pests and malaria. It is a nonpolar chemical pesticide that cannot easily dissolve in water but readily absorbs organic matter such as silt and clay in sediments and soil by hydrophobic bonding. DDT easily dissolves and is stored in the fatty tissue of animals, resulting in bioaccumulation [11,12].

The use of DDT as an insecticide continued for decades after World War II globally [13,14]. The United States banned DDT in 1972 because of its negative impacts on ecosystems and persistence in the environment [15,16]. DDT is still found in biota, sediments, water, and soils across the nation, despite the ban having been enforced for almost 50

* Corresponding authors.
E-mail addresses: girmaebsa4@gmail.com (G. Ebsa), gizachewbirhan@gmail.com (B. Gizaw), mesele2006@gmail.com (M. Admassie), tizazudegu19@gmail.com (T. Degu), asnake.g2008@gmail.com (A. Desalegn), yilma_eshetu@yahoo.com (E. Yilma), wendsenmelak@gmail.com (W. Melak), tadessefm@gmail.com (F. Tadesse), tesfaye.alemu@aau.edu.et (T. Alemu).

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Appendix III Research paper (Published)

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Research article

Screening, characterization and optimization of potential dichlorodiphenyl trichloroethane (DDT) degrading fungi

Girma Ebsa *, Birhanu Gizaw, Mesele Admassie, Asnake Desalegn, Tesfaye Alemu **

Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P. O. Box: 1176, Addis Ababa, Ethiopia

ARTICLE INFO

Keywords:
1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane (DDD)
2,2-bis(p-chlorophenyl)-1,1-dichlorethylene (DDE)
DDT
Degradation
GC-ECD
MALDI-TOF
Optimization

ABSTRACT

Dichlorodiphenyltrichloroethane is an organo-chlorine insecticide used for malaria and agricultural pest control, but it is the most persistent pollutant, endangering both human and environmental health. The primary aim of the research is to screen, characterize, and assess putative fungi that degrade DDT for mycoremediation. Samples of soil and wastewater were gathered from Addis Ababa, Koka, and Ziway. Fungi were isolated and purified using potato dextrose media. Matrix-Assisted Laser Desorption, Ionization, and Flight Duration The technique of mass spectrometry was employed to identify fungi. It was found that the finally selected isolate, AS1, was *Aspergillus niger*. Based on growth factor optimization at DDT concentrations (0, 3500, and 7000 ppm), temperatures (25, 30, and 35 °C), and pH levels (4, 7, and 10), the potential DDT-tolerant fungal isolates were investigated. A Box-Behnken experimental design was used to analyze and optimize fungal biomass and sporulation. The highest biomass (0.981 ± 0.22 g) and spore count (5.60 ± 0.32 log/mL) of *A. niger* were found through optimization assessment, and this fungus was chosen as a potential DDT-degrader. For DDT degradation investigations by *A. niger* in DDT-amended liquid media, gas chromatograph-electron capture detector technology was employed. DDT and its main metabolites, DDE and DDD, were eliminated from both media to the tune of 96–99 % at initial DDT concentrations of 1750, 3500, 5250, and 7000 ppm. In conclusion, it is a promising candidate for detoxifying and/or removing DDT and its breakdown products from contaminated environments.

1. Introduction

The development of agricultural and industrial activities has led to the production and release of numerous artificial and natural substances into the environment [1–3]. The chemical industry manufactures DDT as a synthetic chemical. Such synthetic chemicals are extremely persistent in the environment, as most microbes lack the necessary enzymes for their effective breakdown. However, certain microorganisms have evolved genetic mechanisms for degrading highly persistent DDT compounds [4–6]. DDT is still widely used as an organochlorine pesticide in Asia, Africa, and South America, although it has been banned in a many countries since the 1970s [7–9]. DDT is one of the dangerous persistent chemicals that have harmed biodiversity all over the world.

DDT is one of the dangerous persistent chemicals that have harmed biodiversity all over the world. Even though DDT was banned in

* Corresponding author.
** Corresponding author.
E-mail addresses: girmaebsa4@gmail.com (G. Ebsa), gizachewbirhan@gmail.com (B. Gizaw), mesele2006@gmail.com (M. Admassie), asnake.g2008@gmail.com (A. Desalegn), tesfaye.alemu@aau.edu.et (T. Alemu).

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Appendix IV Research paper (Published)

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Research article

Screening and identification of microbes from polluted environment for azodye (Turquoise blue) decolorization

Birhanu Gizaw *, Tesfaye Alemu, Girma Ebsa

Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, P. O. Box: 1176, Ethiopia

ARTICLE INFO

Keywords:
Azodye
Biolog
Biodecolorization
MALDI-TOF
Mycoremediation

ABSTRACT

Turquoise blue dye is frequently used for industrial dyeing applications. But the release of untreated colored wastewater became an environmental and public health hazard. Microbial remediation of Azodye is environmentally safe and an alternative to a physicochemical approach. The aim of this research is to isolate and characterize turquoise blue dye degrading microbes from polluted environment. Microbial isolation and purification from soil and effluent sample was done on PDA and NA. Turquoise blue dye degrading test was investigated under optimized conditions using -the definitive screening design method. UV–Vis spectrophotometer used to measure the degradation percentage at 620 nm and 25 °C. The results revealed that 24 fungi and 6 bacterial species were identified from the contaminated site using Biolog Microstation and MALDI-TOF. Among all identified microbial species *Penicilium citrinum* Thom BCA & *Penicillium heriquei* show the highest percentage decolorization of turquoise blue dye up to 300 ppm with 90 % removal at pH4 and 87 % at pH 7 up to 400 ppm respectively. The azodye degradation ability of these fungi species used in the development of mycoremediation technologies provide an alternative option for Azodye removal after HPLC analysis, molecular characterization, and toxic analysis.

1. Introduction

Industrial development and rapid population growth have a direct impact on environmental safety. The growth of the global population demanding high quality textile products, thus Azodye containing wastewater, became a significant threat to the environment and water quality [1]. In Ethiopia, the textile sector is the third-largest and fastest-growing manufacturing industry and one of the primary top list source of water pollution [2,3]. According to Ref. [4] research conducted at the Bahir Dar textile industry in Amhara regional state, the liquid waste released from the factory causes significant harm to the aquatic ecosystem and disturbs the downstream users of the Blue Nile River [5]. studied the environmental and health consequences of effluents from textile and garment facilities in Gelan and Dukem of Oromia reginal states, revealing that factories are causing hazards that exceed the federal environmental protection agency's allowed limit (FEPA) for residents and the surrounding environment [6]. Conducted an evaluation of industrial effluent pollution on the Borkena River in Kombolcha Textile, Ethiopia. Pollution levels were over the permitted limits of ambient surface water quality set by the EEPA at the downstream site of the Borkena River (see Table 6).

Synthetic Azo dye was widely used in various industries such as textile, printing, paper, tannery, pharmaceutical, food, cosmetics and manufacturing, accounting for more than 70 % of total dye usage with a global production of 1,000,000 tons [7–10]. During the

* Corresponding author.
E-mail address: gizachewbirhan@gmail.com (B. Gizaw).

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Appendix V Research paper (Published)

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Fungi species identified from polluted environment for chromium sequestration and solid state fermentation on tannery shaving waste

Birhanu Gizaw [a, b, *], Tesfaye Alemu [b, **], Girma Ebsa [b], Zerihun Tsegaye [a]

[a] Ethiopian Biodiversity Institute, Microbial Biodiversity, Addis Ababa, P.O.Box: 30726, Ethiopia
[b] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, P. O. Box: 1176, Ethiopia

ARTICLE INFO

Keywords:
Chromium
Degradation
FAAS
MALDI-TOF
Optimization

ABSTRACT

Accumulation of hexavalent chromium in the environment affects our ecosystem. Physicochemical treatment did not solve the problem, due to high requirement of operational costs & energy, insolubility of Cr (VI) at any pH & sludge accumulation. The study aim is screening and evaluating fungi for chromium biosorption for mycoremediation. Soil and effluent sample were collected. PDA media was used for fungi purification. Fungal chromium tolerance evaluation and optimization was conducted at pH (4, 7, 10), T^0 (25, 27.5, 35 °C), Cr concentration (5000–25000 ppm) using Full Factorial experimental design. Spore count, biomass production, Cr tolerance index, mycelia growth were evaluated. Flame Absorptive Spectrophotometer was used for quantification of fungal chromium removal. Fungi species were identified by MALDI-TOF. Fungi growth & degradation of tannery shaving waste was evaluated. The result revealed 6 potential fungi, *T. longibrachiatum, T. koningii, A. niger, G. candidum, P. rubens, T. orientale* were identified. Optimium mean biomass & spore count were 0.514 ± 0.051& 1.39 ± 0.038 at pH4 and 27.5 °C respectively. MTL test indicate that *Trichoderma* species, *A. niger* & *P. rubens* survive up to 10,000 ppm, 15, 000 ppm & 20,000 ppm respectively. FAAS analysis of fungi chromium removal from aqueous media result indicate that *A. niger* (99%). *T. longibrachiatum* (85%) and *P. ruben* (84.6%). These 6 fungi are efficient in tannery shaving waste degradation but *A. niger* was superior in spore count 15.1 Log/mL and 7 g substrate weight loss. In conclusion three fungi have high chromium tolerance and removal capacity and shaving waste degradation promising for mycoremediation.

1. Introduction

The geochemical process, agrochemical application, industrialization and anthropogenic activities promote chromium accumulation in environment (Bharti and Sharma, 2022; Shahid et al., 2015). The global environment has contaminated with more than 1.29×10^5 tons of chromium (Coetzee et al., 2020). Tannery industries are the major sources of chromium pollution. Tanning factories generate approximately 40 million m^3 of Cr-containing waste water annually on a global scale (Arishi and Mashhour, 2021). Currently, in Ethiopia, more than 30 tannery industries are found and approximately 31.4 million kg of skin are produced annually. The study indicate that the yearly volume of liquid waste discharge from 15 tanneries in Ethiopia is expected to produce between

* Corresponding author.
** Corresponding author.
E-mail addresses: gizachewbirhan@gmail.com (B. Gizaw), tesfaye.alemu@aau.edu.et (T. Alemu).

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Appendix VI Research paper (Published)

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RESEARCH

Isolation of Hexavalent chromium tolerant fungal species from urban vegetable farm soil and effluent waste in Addis Ababa& Rift valley, Ethiopia

Birhanu Gizaw[1] · Tesfaye Alemu[2] · Girma Ebsa[2] · Dinkitu Wako[1]

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Abstract
Hexavalent chromium is resistant to degradation and harmful toxic substance to environment and community health. Physicochemical treatment methods are demanding high cost, used large quantities of chemicals & energy, release large amount of secondary toxic degradants. Mycoremediation is an eco-friendly alternative treatment method. The main objective of this research is to isolate and characterize chrome (VI) tolerant fungi from farm soil & industry effluent for mycoremedation purpose. The screening and isolation of yeast was carried out on potato dextrose agar media. PDA and broth assay test for fungi tolerance to hexavalent chromium at different concentration, temperature and pH was evaluated. Fungi species was identified biochemically using Biolog Microstation depending on carbon utilization and chemical sensitivity test. The result revealed that 10 yeast species was identified with full ID from effluent waste and farm soil based on their probability ≥ 75% and similarity index ≥ 0.5 as well as their Cr (VI) tolerance ability up to 2500 ppm. These are *Yarrowia lipolytica* (100%, 0.7), *Cryptococcus luteolus*(100%, 0.64), *Rhodotorula aurantiaca* A(100%, 0.62), *Ustilago maydis*(100%, 0.58) *Trichosporon beigelii* B (100%, 0.51), *Cryptococcus terreus* A (100%, 0.62), *Zygosaccharomyces bailii* (98%, 0.65), *Nadsoniafulvenscens* (90%, 0.62), *Schizoblastosporonstarkeyihenricii* (89%, 0.56), *Endomycopsis vivi* (84%, 0.62), *Rhodotorula pustula* (Sim, 0.59). Two yeast species *Yarrowia lipolytica* and *Nadsoniafulvenscens* show the highest growth mean Optical density (OD) measure 0.74 ± 0.2 & 0.60 ± 0.2 respectively at pH 7 & 25 °C. The highest tolerance index (mm) was recorded by *Schizoblastosporon starkey henricii* 0.3067 ± 0.152. Cr (VI)-tolerance ability of these yeast strains used in the development of chromium-bioremediation technologies provide an alternative option for chromium sequestration after HPLC analysis& molecular characterization.

Keywords Biolog · Chromium · Hexavalent · Mycoremediation · Physico-chemical · Yeast

Introduction

Environmental pollution and industrial development have been major issues for decades. One of the many harmful heavy metals generated by anthropogenic and industrial activity that harms terrestrial and aquatic ecosystems is hexavalent chromium (Cr (VI). Chromium waste is produced by a variety of sectors, including textile, tannery, electroplating, dye and metallurgy (Nakkeeran et al. 2018; Lian et al. 2019). Today, more than 27 tanneries exist in Ethiopia that export semi-finished and finished leather and a few more are under construction (Coppeaux et al. 2016). In Addis Ababa and other parts of Ethiopia, factories built close to rivers discharge uncontrolled and untreated effluent waste water into the waterways (Gebre and Van Rooijen 2009). In turn, Addis Ababa and the surrounding area's urban vegetable growing operations use this waste water for direct irrigation. According to CSA (2017), the percentage of water bodies with no chemical contamination is projected to be around 13%. Chemical contamination in water bodies is relatively significant. Chromium pollution are the most serious environmental problems specially in farm land, groundwater, surface water and community health due to urbanization,

✉ Birhanu Gizaw
gizachewbirhan@gmail.com

[1] Microbial Biodiversity Directorate, Ethiopian Biodiversity Institute, P.O. Box 30726, Addis Ababa, Ethiopia

[2] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P. O. Box: 1176, Addis Ababa, Ethiopia

Appendix VII Review paper (Published)

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journal homepage: www.elsevier.com/locate/btre

Review

The role and mechanisms of microbes in dichlorodiphenyltrichloroethane (DDT) and its residues bioremediation

Girma Ebsa [a,*], Birhanu Gizaw [a,1], Mesele Admassie [a,1], Tizazu Degu [b,1], Tesfaye Alemu [a,1]

[a] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P. O. Box: 1176, Addis Ababa, Ethiopia
[b] Department of Crop Protection, Ethiopian Institute of Agricultural Research, P. O. Box: 2003, Addis Ababa, Ethiopia

ARTICLE INFO

Keywords:
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Dichlorodiphenyldichloroethylene
Dichlorodiphenyltrichloroethane
Microbial remediation
Soil
Water

ABSTRACT

Environmental contamination with dichlorodiphenyltrichloroethane (DDT) has sever effects on the ecosystem worldwide. DDT is a recalcitrant synthetic chemical with high toxicity and lipophilicity. It is also bioaccumulated in the food chain and causes genotoxic, estrogenic, carcinogenic, and mutagenic effects on aquatic organisms and humans. Microbial remediation mechanism and its enzymes are very important for removing DDT from environment. DDT and its main residues dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) can biodegrade slowly in soil and water. To enhance this process, a number of strategies are proposed, such as bio-attenuation, biostimulation, bioaugmentation and the manipulation of environmental conditions to enhance the activity of microbial enzymes. The addition of organic matter and flooding of the soil enhance DDT degradation. Microbial candidates for DDT remediation include micro-algae, fungi and bacteria. This review provide brief information and recommendation on microbial DDT remediation and its mechanisms.

1. Introduction

The main drivers of environmental contamination include modern farming methods, industrialization, fast urbanization, and other human anthropogenic activities [1]. These activities release a significant amount of toxic pollutants into the environment and affect both animals and plants in the corresponding ecosystems [2–6]. It was the first synthetic insecticide chemical used all over the world. It has been used as an insecticide to combat pests in agriculture and diseases like malaria and typhus [7–9]. Recently, most nations banned the use of DDT because of its harmful effects on animals and human health through the food chain. DDT may lead to a variety of acute and chronic diseases in humans [10, 11].

According to many research reports, less than 1 % of all DDT pesticides used are utilized to target pests; the remaining DDT pesticides are precipitated in the nearby water and soil. The remaining DDT in the ecosystem has a negative impact on the ecology [12,9]. DDT and its residuals have been found in both industrialized and developing nations' environments [13,14]. Due to its toxicity and persistence in the environment, environmentalist and health administrators, as well as members of the general public, have voiced concerns [15–17]. The breakdown of DDT insecticides frequently produces both toxic and non-toxic intermediates, which should be taken into consideration when developing a remediation strategy. Microbes, such as fungi, bacteria, microalgae, and others, are reported for DDT breakdown and used as bioweapons to fight DDT chemicals and caught the researcher's attention in recent years [18,19]. DDT degradation is dependent on natural reactions in the environment, such as chemical, biological, and physical. These reactions take place when a DDT pesticide particle interacts with a soil particle, a water molecule, or a living organism [20–22]. Abiotic and biotic components, as well as the physical and chemical characteristics of the soil, all have an impact on the rates of DDT degradation in soil and water [23,22]. In DDT degradation process, the presence of soil and water microbes is a very important factor, which depends on the environmental conditions of the soil and water [24,25].

DDT can be eliminated from soil and water using a variety of microbial remediation technologies [23,26]. Bacterial remediation of DDT in contaminated water and soil is based on the capability of bacterial cells to tolerate and accumulate DDT pollutants. The bacterial population and DDT content determine the rate of degradation [27,28]. Both aerobic and anaerobic conditions allow bacteria to degrade DDT. Some reported bacterial strains with degradation potential are Escherichia

* Corresponding author.
E-mail address: girmaebsa4@gmail.com (G. Ebsa).
[1] Co-authors

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Mini Review

Microbial Remediation of Dichloro-Diphenyl-Trichloroethane (DDT)

Girma Ebsa*, Tesfaye Alemu and Birhanu Gizaw

Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia

***Corresponding author:** Girma Ebsa, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia

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Abstract

The insecticide had been intensively applied for agricultural pest control since 1940. It was disqualified because it persists in the environment, accumulates in fatty tissues, and can cause bad health effects on wildlife and human being. The organochlorine (DDT) has been programmed under the Stockholm Convention to protect human health and the environment from Persistent Organic Pollutants (POPs). Biodegradation is carried out by microorganisms (bacteria and fungi) that naturally live in the environment. Bacteria and fungi have very diverse metabolisms, and they use a wide variety of food and energy sources and perform many important functions by decomposition. Complete biodegradation of DDT involves the oxidation of parent compound to form carbon dioxide and water and provides both carbon and energy for the growth and reproduction of microbes. Each degradation step is catalyzed by specific enzyme produced by a degrading cell or enzyme found external to the cell. Degradation of insecticide by enzyme will stop at any step if an appropriate enzyme is not present. Effects of DDT on human health and the environment depend on the dose of DDT and the timespan and frequency of exposure. It effects also depend on the health of a person and certain environmental factors. DDE and DDT can pass to the fetus in pregnant women. Both chemicals are found in breast milk, resulting in exposure to nursing infants. Microbes can be screened out from soil and wastewater as an effective tool for biodegradation of toxic organic chemicals. Phanerochaete and related fungi that have the ability to attack wood possess a powerful extracellular enzyme that, acts on a broad array of organic compounds.

Introduction

Ethiopia is a country of more than 1.1 million square kilometers, located in the Horn of Africa between approximately 40 and 150 north latitude and 320 and 490 east longitude. Ethiopia has rich in biodiversity of animals, plants and microorganisms where overuse of pesticides has adverse effects on the loss of natural recourses. The Ethiopian government identified the impact of these measures in preparation of the Conservation Strategy of Ethiopia (1997) and becoming a signatory in 1992 and ratifying the Convention on Biological Diversity (CBD) in 1994 (Et-NBSAP, 2015). In Ethiopia and worldwide, farmers used DDT on a variety of food crops. DDT is a practicable insecticide in indoor residual spraying owing to its effectiveness in well supervised spray operation and high excito-repellency factor. Although DDT is very effective in killing or repelling mosquitoes its use has been severely reduced and restricted to indoor residual spraying, due to its persistence in the environment and ability to bioconcentrate in the food chain [1,2]. DDT was so broadly used because of effective, relatively inexpensive to manufacture, and lasts a long time in the environment [3]. It was disqualified because it persists in the environment, accumulates in fatty tissues, and can cause bad health effects on wildlife [4]. Resistance occurs in some insects (like the house fly) that develop the ability to quickly metabolize the DDT (WHO, 1979). DDT affects the nervous system by interfering with normal nerve impulses [3]. The organochlorine (DDT) has been programmed under the Stockholm Convention to protect human health and the environment from Persistent Organic Pollutants (POPs). The Convention aims to decrease and ultimately eradicate DDT but recognizes the acceptable production and use of DDT for disease vector control. DDT is one of the insecticides recommended by WHO for indoor residual spraying for malaria control (ATSDR, 2002). The Stockholm Convention has been established for DDT register in which countries are appreciated to report their intention to produce or use DDT. The continued need for DDT disease vector control, which is subject to evaluation by the conference of the parties during its regular meetings held every 2 years, was confirmed in 2015. Changes in DDT levels in humans and the environment have