Mutagenic Effects of Pesticides on Enteric Bacteria and Their Role in the Rise of Antimicrobial Resistance (AMR).

By

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A thesis submitted to the Department of Mathematics & Natural Sciences in partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology

Department of Mathematics & Natural Sciences

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Declaration

It is hereby declared that

1. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
2. I have acknowledged all main sources of help.

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Approval

The thesis titled "Mutagenic Effects of Pesticides on Enteric Bacteria and Their Role in the Rise of Antimicrobial Resistance (AMR) Globally" submitted by Somnath Gupta. ID: 22376009 of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters of Science in Biotechnology on 14th December 2023

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Ethics Statement

I hereby declare that I did not conduct any sort of unethical action whatsoever as a means to perform my thesis work. I remained wholeheartedly sincere, honest and worked with utmost dedication to achieve this work. No living organism was hurt or killed while conducting this research.

Abstract/ Executive Summary

Microbial antibiotic resistance threatens world health dangerously. Moreover the non-antibiotic stressors have also contributed to bacterial antibiotic resistance. Pesticides affect bacterial antibiotic resistance, and this study seeks to understand the process. Efflux pumps has been activated by pesticide stress and induces antibiotic-resistant gene alterations in bacteria. Pesticides increase cell membrane permeability and bacterial mobile gene elements, which increases antibiotic resistance gene transmission. Despite multiple studies linking mutagenic effect of pesticides on enteric bacteria, a comprehensive review is lacking. For this study a systematic search was performed using four databases (PubMed, Scopus, Web of Science and Embase) and one search engine (Google Scholar) for original studies (From February 2022 to April 2023). Later, between July 2022 and August 2023, the findings were cross-checked and updated the existing literature. As per our eligible criteria, overall 101 studies were selected for the analysis. Throughout this study 471 pesticide chemicals have been identified from 101 publications, and a significant portion of them are mutagenic to *Salmonella typhimurium, Escherichia coli,* and *Bacillus subtilis*. Many pesticide mutagenicity investigations use *Salmonella* as the main testing strain. Studies have found that *Salmonella* strains TA100, TA98, and TA1535 are used most often, with *E. coli* strain WP2 seldom used. The Ames test is usually considered a reliable mutagenicity test. Mutagenic doses vary with organism and strain spanning from the range of 0.1 ml/plate to 5000 µg/plate. Acephate (0.1 µg/plate), Allethrin (2000 µg/plate, 1500 µg/plate, 0.1 µg/plate), Demond EC 25 (400 and 800 µg/plate), Dicrotophos (5000 µg/plate), and Lambda-cyhalothrin (5, 10 µmol/plate, 0.5, 1, 2.5, 5, and 10 µmol/plate) are some of the insecticidal compounds that mutate *S.* Typhimurium strains at different doses. Moreover, dichlorvos showing mutagenicity on *E. coli* at 0.1 ml/plate doses and Dibrom showing mutagenicity on *B. subtilis* at 50,100 and 300 µg/plate. Similarly, some of the fungicidal compounds like Thiram, TBZ, NNN, Folpet showing mutagenicity on different organisms at different doses. The doses are (0.05 and 0.5 mg/plate, 0.01, and 0.1 mg/well, 200µg/plate, 50 µg/plate, 50 µg/plate, 1 µg/plate), respectively. And for herbicides, HEH (2-hydrazinoethanol), Roundup, Triallate poses mutagenicity on different organism at 0.1ml/plate, 720 µg/ plate and 50, 100, and 300 µg/plate, respectively. Most concerning is the study's link between mutagenic chemicals and human and animal food samples. The US Food and Drug Administration found pendimethalin, a moderately hazardous herbicide, in animal and human food samples. Additionally, the food samples contain high levels of dichlorvos, carbofuran, and monocrotophos, which are harmful to human health. Heptachlor, an outdated

insecticide, was found in food samples. This comprehensive study shows the mutagenic effects of pesticides that encompasses total 23% of the compounds that showing mutagenicity. Among them insecticides showing mutagenicity 96.67 % (58 out of 60) in *S.* Typhimurium, along with 50% on *E. coli* (30 out of 60) and 1.67% on *B. subtilis* (1 out of 60). Out of 26 mutagenic compounds, fungicides showing mutagenicity on *S.* Typhimurium, *E. coli* and *B. subtilis* at 96.15%, 34.61% and 7.7%, respectively. For herbicide out of 20 mutagenic compounds 20 (100%) shows mutagenicity for *S.* Typhimurium, 2 of them (10%) shows mutagenicity on *E. coli* and one of them (5%) shows mutagenicity on *B. subtilis.* These mutagenic pesticides have also been found in human and animal food samples. Overall exposure and mutagenic impacts of the pesticides could generate a potential links to raise antibiotic-resistant microorganisms.

Keywords: Antibiotic Resistance, Antibiotic Resistance Genes, Pesticides, Mutagenicity, Mutagenic Doses, Different Strains, Hazardous, Human and Animal Food Sample.

Dedication

This thesis is dedicated to all my struggles and hard times that pull me back but never be able to make me stop.

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List of Acronyms

AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistance Genes
ARB	Antibiotic-Resistant Bacteria
ACh	Acetylcholine
WHO	World Health Organizations
FDA	Food and Drug Administration
TB	Tuberculosis
MDR	Multi Drug Resistance
TDR	Total Drug Resistance
MRSA	Methicillin-resistant *Staphylococcus aureus*
CDC	US Center for Disease Control and Prevention
PRISMA-ScR	Preferred Reporting Items for Systematic Review and Meta-Analyses extension for Scoping Reviews
HGT	Horizontal gene transfer
IRAC	The Insecticide Resistance Action Committee
MIC	Minimal inhibitory concentration
DNA	Deoxyribonucleic acid
WGS	Whole Genome Sequencing

CHAPTER 1

INTRODUCTION

The emergence and proliferation of antibiotic resistance among microorganisms pose a significant and escalating threat, particularly within the realm of clinical medicine. This phenomenon is distinguished by the capacity of microorganisms to acquire resistance to an extensive array of frequently employed antibiotics, has become increasingly prevalent and worrisome. As the effectiveness of existing antibiotics diminishes, the medical community faces mounting challenges in combating infectious diseases and maintaining successful treatment outcomes. It is evident that a significant number of bacterial species have developed tolerance towards these antibiotics, rendering them ineffective in contemporary clinical settings. The emergence of antibiotic resistance has posed a significant challenge in the field of medicine. Despite the development of alternative antibiotics, bacteria have persistently demonstrated their ability to adapt and defend against these new classes of substances. This ongoing battle between bacteria and antibiotics has necessitated a deeper understanding of the mechanisms underlying bacterial resistance.

1.1 Antimicrobial Resistance

Antibiotics are pharmaceutical substances employed for the purpose of treatment of bacterial illnesses. Antibiotic resistance is a phenomenon that arises when bacteria undergo alterations in reaction to the administration of certain pharmaceutical agents. Antibiotic resistance is primarily observed in bacteria rather than in humans or animals. These bacteria have the potential to infect both humans and animals, and the resulting diseases are more challenging to manage compared to those produced by germs that do not possess resistance.

Antimicrobial resistance (AMR) has become a significant concern in the 21st century, posing a threat to the successful prevention and treatment of a wide range of infections caused by bacteria, parasites, viruses, and fungi. These microorganisms have developed resistance to commonly used medications, making them increasingly difficult to treat. Addressing antibiotic resistance in bacteria is a pressing issue, particularly in the context of AMR. Throughout the years, bacteria causing both common and severe infections have gradually developed resistance to every new antibiotic that has been introduced to the market. Given the current circumstances,

it is absolutely crucial to take immediate action in order to prevent a potential global health care crisis. [1]

Also, some forms that are resistant to multiple drugs (MDR) emerged. Because they have gone through many changes, so-called "superbugs" are very resistant to different types of antibiotics. They are responsible for a lot of illness and death, and treating them is hard, which means longer hospital stays and higher healthcare costs. Multidrug resistance is still a problem in the treatment of tuberculosis (TB), which is the most common human pathogen. For example, it is now necessary to use a mixture of anti-TB drugs to treat TB. Some forms of Mycobacterium tuberculosis are very resistant to four or more of the first-line drugs (XDR strains) [2,3] and It gets even worse; types that are completely drug-resistant (TDR) have been found.[4]

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are another threat. These strains are not only resistant to methicillin (which was created to treat penicillinase-producing *S. aureus*), but they may also shows resistant to aminoglycosides, tetracyclines (TET), macrolides, chloramphenicol (CHL), lincosamides (LIN), and disinfectants. They are becoming extensively linked to hospital-acquired infections. Vancomycin (VAN) is the last resort for these types of strains that are resistant to other drugs. However, *Enterococcus* species are often resistant to VAN, which is scary because it can be passed on to MRSA strains through horizontal gene transfer (HGT). Vancomycin-resistant MRSA forms have already been found, but they are still very rare. [5]

1.2 Impacts of Antimicrobial Resistance:

The mortality and public health cost of antibiotic resistance are hard to measure, and few research address this topic. The US Center for Disease Control and Prevention (CDC) estimates that antibiotic-resistant illnesses impact over 2 million people annually, resulting in at least 23,000 deaths [1]. “According to recent estimates, in 2019, 1.27 million deaths were directly attributed to drug-resistant infections globally. By 2050, up to 10 million deaths could occur annually”.[6]

In 2007, the number of infections and deaths caused by multidrug-resistant bacteria (*Staphylococcus aureus, Escherichia coli, Enterococcus faecium, Streptococcus pneumonia, Klebsiella pneumonia and Pseudomonas aeruginosa)* in Europe was estimated at ∼400 000 and ∼25,000, respectively. [7]

Moreover, without good antibiotics, cancer treatments, organ transplants, hip replacement surgery, intensive care for pre-term neonates, and other medical procedures would be impossible. Multidrug-resistant bacterial infections are a major source of morbidity and mortality in these treatments. [1] A recent study from the Medical University of Warsaw, on infections after orthotopic liver transplantation, showed a high proportion of isolates of antibiotic-resistant bacteria. [8]

Improper use of medicines results in a rise in antibiotic resistance. Public health is seriously threatened by infections produced by gram-negative bacteria that are resistant to antibiotics. A study from national data on antibiotic consumption from 2009 to 2015, Point Prevalence Survey data on inpatient antibiotic usage from 2015, and national AMR data on 79 *Klebsiella pneumoniae* and 68 *Escherichia coli* isolates collected from 8 hospitals between 2016 and 2018. The prevailing antibiotics utilised were third-generation cephalosporins, with a median yearly consumption rate of 0.66 DDD/1000/day. Ceftriaxone was the predominant antibiotic recommended for the treatment of both community and hospital acquired infections, as well as for surgical and medical prophylaxis. The resistance rates were highest for *Klebsiella pneumoniae* against ceftriaxone, ceftazidime, and gentamicin (93.59%, 90.79%, and 89.87% respectively), and for *Escherichia coli* against aminopenicillins, ceftriaxone, and ceftazidime (89.06%, 70.15%, and 61.54% respectively). The excessive use of broad-spectrum antibiotics in Montenegro is correlated with the elevated prevalence of resistance in *Klebsiella pneumoniae* and *Escherichia coli* towards these medications. The development of an antimicrobial stewardship programme is necessary in Montenegrin hospitals to address the issue of antibiotic overuse. [9]

1.3 Factors Influencing Antimicrobial Resistance:

A lot of unsolved puzzled behind the increase of antimicrobial resistance bacteria in the environment. Among them antibiotic consumption in the community and in hospital settings, Incorrect knowledge about antibiotics in the population and self-medication, misuse and over use of antibiotics, poor sanitation and Uncontrolled pesticide uses and its mutagenic effects are most concerning. [10]

Misuse of antimicrobials is the primary cause of AMR presence and development. Human usage of antibiotics (underuse, overuse, and abuse) causes selective pressure, not natural processes. Unfortunately, excessive usage of these compounds caused severe environmental harm. Since their debut, millions of tonnes of antibiotics have been manufactured, causing environmental toxicity and promoting resistant microbial populations. [11]

When compared to the medical sector, the prevalence of resistant bacteria in agriculture constitutes a comparatively insignificant portion of the global reservoir of microbial resistance. . However, it serves as a noteworthy illustration of how prolonged exposure of microorganisms to antibiotics creates a selective force that enables certain bacteria to adapt to environmental growth-inhibiting agents. The presence of transposons or other protective mechanisms in microorganisms enables their survival and proliferation, leading to the emergence of multi-resistant bacteria in the surrounding environment. In addition, these microbes exhibit limited methods via which they can completely neutralize the effectiveness of antibiotics. These mechanisms include the presence of β-lactamases, acetylates specific to chloramphenicol, esterases for macrolides, and aminoglycoside-inactivating enzymes. Through alternative means, the antibiotic is discharged from. The cellular structure remains intact, without undergoing destruction. The substance is exclusively discharged into the surrounding ecosystem, where it persists and exerts its selective influence through gradual accumulation. At low concentrations, which are ideal for the purpose of selecting resistant strains. [12]

The soil microbiota plays a crucial role in the early development of antimicrobial resistance AMR and serves as a reservoir of genes that confer resistance to clinically relevant infections. Metagenomics investigations have successfully detected the transfer of antibiotic resistance genes (ARG) between bacteria in the environment and pathogens seen in clinical settings The soil bacteria that are resistant to many drugs, commonly referred to as multidrug-resistant (MDR) bacteria, include genetic elements that confer resistance to various classes of antibiotics, including amphenicols, aminoglycosides, β-lactams, sulfonamides, and tetracycline. [13]

Another alarming and rarely noticed factor that influences AMR is the use and effects of pesticides in agricultural field. Co-resistance occurs when a pest becomes resistant to multiple pesticides due to a genetic change, frequently with a similar mode of action. [14] This phenomenon can occur between pesticide classes, heavy metals, and antibiotics, and may be mediated physiologically (cross-resistance) or genetically (co-resistance). Co-resistance occurs

when nearby related resistance genes on a mobile genetic element are present. Bacteria grow tolerant to antibiotics and heavy metals through cross-resistance. [15] There are multidrug efflux pumps that rapidly eliminate harmful agents (antibiotic/metallic ions) from the cell. [16]

Various pests, such as insects, rodents, fungus, and weeds, can be effectively eradicated through the utilization of pesticides. Currently, the number of pesticides employed exceeds one thousand, encompassing a wide range of distinct types. Pesticides are employed in the field of public health to eradicate disease-transmitting insects such as mosquitoes, while in the agricultural sector, they are utilized to eliminate pests that cause damage to crops.

In addition to antibiotics, microbes must also defend against other substances. Insecticides and herbicides introduced in the 1940s and 1950s led to the development of resistant plant and insect species. Pesticide resistance refers to a pest population's reduced susceptibility to previously effective pesticides. The mechanism of pesticide resistance is hereditary. Pesticides kill most pests in a population, but some may persist. Higher concentrations or frequent applications fall short of killing the entire population. The offspring of pests that survived will be able to resist pesticides like their parents. This process occurs with each generation, and as most pests have a new generation within weeks, increasing application frequency leads to more resistant pests, ultimately causing the entire population to become resistant. [17]

Pesticide resistance is defined by the Insecticide Resistance Action Committee (IRAC) as a heritable alteration in the sensitivity of insect populations to pesticides., resulting in repeated pesticide failure to achieve optimal control when administered as recommended for the species. Pests currently pose a significant threat to human health, particularly in medical and agricultural industries. For instance, pests that are resistant to pesticides can spread human illnesses that are resistant to conventional disease treatments. Recent studies show that insecticides are now ineffective against over 500 insect and mite species, Two hundred and Seventy weed species, One hundred and Fifty plant diseases, and several rat species. Similar to antibiotics, increased pesticide resistance from several chemical classes was observed. [18]

Herbicides are utilized globally and can be found in food, humans, the environment, pets, and residences. Although these compounds are initially designed to harm plants, their application in agricultural crops or gardens increases the risk of pathogen exposure for both humans and animals. Herbicides are typically examined for toxicity, but not for sub lethal effects on microorganisms, which may lead to antibiotic tolerance. [19] The exposure of *E. coli* and *S.* Typhimurium to three herbicides: dicamba (3,6-dichloro-2-methoxyben-zoic acid; Kamba),

2,4-D, and glyphosate (N-(phosphonomethyl) glycine; Roundup), as well as salicylic acid, which shares structural similarities with the first two has been studied. The study found that these strains' behavior changed in response to various antibiotic classes, including ampicillin (AMP), ciprofloxacin (CIP), kanamycin (KAN), tetracycline (TET), and chloramphenicol (CHL). Results varied by species, herbicides, and antibiotics. *S.* Typhimurium exposed to 2,4-D showed enhanced tolerance to AMP, Complementary and Alternative Medicine (CAM), CIP, and TET but increased susceptibility to KAN. Roundup dramatically enhanced KAN and CIP tolerance. Exposure to Kamba and 2,4-D had similar effects on *E. coli*, but did not improve AMP tolerance. After exposure to Roundup, tolerance to KAN and CIP increased. A significant effect was observed when strains were simultaneously exposed to antibiotics and herbicides. This may be due to changes in target exposure to the antibiotic due to changes in efflux or permeability. Activating the AcrAB-TolC efflux pump in *E. coli* and *S.* Typhimurium reduces resistance to fluoroquinolones, lactams, TET, and CHL. Salicylic acid, a recognized inducer of AcrAB-TolC, can increase susceptibility to aminoglycosides. While exposure to Kamba or 2,4-D increases susceptibility to KAN, exposure to Roundup decreases susceptibility to aminoglycosides. Herbicides can increase antibiotic minimal inhibitory concentration (MIC) values by up to 3 times, considerably impacting bacterial infection treatment. [19]

Recent findings link agricultural fungicide use to the presence of triazole-resistant *Aspergillus fumigatus* in azole-naive individuals. The isolates with the TR34/L98H and TR46/Y121F/T289A mutations in the CYP51A gene and promoter area showed resistance in both environmental and clinical samples from Europe, Asia, and Africa. However, several clinical strains from the US and Latin America lack fungicide-driven resistance in *A. fumigatus*, despite widespread pesticide usage in the region. In a 2015 study, 60 soil samples from flower fields and greenhouses in Columbia were examined. *Aspergillus* strains were evaluated for azole resistance on media containing 4 mg/L itraconazole or 4 mg/L voriconazole. Twenty *A. fumigates* strains resisted. CYP51A gene changes were examined. [20]

1.4 Pesticides and Its Classification:

Pesticides are substances, either chemical or biological in nature, that are employed to manage and regulate populations of pests, including insects, weeds, rodents, and fungus. They are employed in diverse contexts, encompassing agricultural, forestry, urban areas, and residential environments.

Pesticides can be categorized in several ways, but the most prevalent classification is based on the specific pest they are intended to eradicate. The primary categories of pesticides include:

Table 1 Pesticides and its classifications based on pest

Class	Type of pest	Uses
Insecticides	Insects	Used to control a wide variety of insects, including mosquitoes, flies, cockroaches, ants, termites, and beetles.
Herbicides	Weeds	Used to control weeds in crops, lawns, and other areas.
Fungicides	Fungi, mold, and mildew	Used to control fungi, mold, and mildew on crops, fruits, vegetables, and other plants.

Pesticides can also be categorized based on their mode of action, which refers to the specific way in which they function to eliminate or manage pests. Contact pesticides are a type of pesticide that effectively eliminate bugs upon direct exposure to the pesticide. Systemic pesticides are a type of pesticide that is taken up by the plant or pest and distributed throughout the organism, resulting in its death.

Pesticides are extensively employed in agriculture to safeguard crops against pests and illnesses. Additionally, they are employed in the field of forestry to manage pests that have the potential to cause harm to trees and other resources within the forest. Pesticides are employed in urban areas to manage pests such as mosquitoes, cockroaches, and ants. Pesticides are employed within residential settings to manage pests such as termites, fleas, and ticks.

While pesticides can effectively manage pests, they can also exert adverse effects on human health and the environment. Using pesticides in a safe and responsible manner, while adhering to all label instructions, is crucial. [17]

1.4.1 Mutation

A mutation refers to an alteration in the genetic sequence of an organism's DNA. Mutations may arise due to inaccuracies in DNA replication during cellular division, exposure to mutagenic agents, or as a consequence of viral infections. Germline mutations, which manifest

in eggs and sperm, have the potential to be inherited by subsequent generations, but somatic mutations, which arise in body cells, are not heritable.

Cellular mutations occur continuously inside our biological systems; yet, the overwhelming majority of these mutations have negligible impact on our overall health. There exist numerous factors that often mitigate the significant implications of mutations. One compelling factor lies in the intricate machinery present within our cells, enabling prompt and efficient correction of mutations. The limited time available to them precludes the possibility of them causing any issues. Another notable observation is that the majority of mutations mostly manifest in somatic cells, such as muscle cells or skin cells. These mutations are limited in their impact, as they solely influence the specific cell in which the mutation originated and any subsequent cells that derive from it. Conversely, in instances where mutations manifest in germline cells, namely eggs and sperm, they will be uniformly inherited by all subsequent cells derived from the fertilized egg, encompassing the complete individual, and potentially yielding more substantial consequences. [21]

1.4.2 Mutagenicity of Pesticides and Its Impact on Antimicrobial Resistance (AMR)

Some pesticides have been shown to be mutagenic in laboratory studies. For example, the pesticide glyphosate has been shown to cause deoxyribonucleic acid (DNA) damage in human cells. Other pesticides, such as malathion and chlorpyrifos, have also been linked to mutagenicity in animal studies. [22]

One of the ways that pesticides can contribute to AMR is by causing mutations in bacteria. These mutations can allow bacteria to develop resistance to antimicrobial drugs. For example, a study published in the journal Environmental Science & Technology found that exposure to the pesticide chlorpyrifos could make bacteria resistant to the antibiotic ciprofloxacin.

Another way that pesticides can contribute to AMR is by killing off beneficial bacteria. Beneficial bacteria, such as those in the gut microbiome, play an important role in protecting us from infection. When pesticides kill off these bacteria, it can make us more susceptible to infection by antibiotic-resistant bacteria (ARB). [23]

The mutagenic effects of pesticides are a serious public health concern. These effects can have a significant impact on AMR, which is already a major global problem. It is important to take

steps to reduce exposure to pesticides, such as choosing organic foods and using non-toxic pest control methods.

1.5 Salmonella/microsome Mutagenicity Assay (AMES) Test

The Ames test is a bacterial assay that detects possible mutagens by reverse mutation. The test is both straightforward and efficient, capable of analyzing a diverse range of compounds such as chemicals, food additives, and environmental materials.

The Ames test is predicated on the idea that mutagens have the capability to induce bacteria to transition from a mutant state to a wild-type state. Reversion takes place when the mutagenic agent impairs the DNA of the bacteria, resulting in an alteration in the DNA sequence. If this alteration occurs in the gene accountable for the mutation, the bacterium will revert to the original form.

The Ames test is conducted utilizing a distinct strain of bacteria known as *S.* Typhimurium. The bacteria are cultivated in a culture medium and subsequently subjected to the substance under investigation. In the event that the chemical exhibits mutagenic properties, a portion of the bacteria will undergo a reversion to their original, non-mutated form. Subsequently, the count of revertants is conducted, and this count is employed to ascertain the mutagenic capacity of the material.

The Ames test is an exceedingly sensitive assay capable of detecting even minute quantities of mutagens. Additionally, this test is highly dependable and yields highly consistent findings. The Ames test is extensively employed to detect possible mutagens across various contexts because to these justifications. [24]

1.6 Whole Genome Sequencing (WGS) Methods

The laboratory procedure known as whole-genome sequencing (WGS) is utilized to ascertain the precise sequence of bases (A, T, C, and G) in the complete genome of an organism. This comprehensive examination offers a thorough depiction of an organism's genetic composition, facilitating the identification of genetic variants, mutations, and structural rearrangements within its DNA. WGS has significantly transformed our comprehension of biological processes and has been extensively utilized across several disciplines.

The utilization of WGS is progressively being employed within therapeutic contexts for the purpose of diagnosing genetic illnesses, determining susceptibilities to specific diseases, and informing individualized treatment strategies. Through comprehensive examination of an individual's complete set of genetic material, medical professionals are able to identify precise genetic alterations that have a role in the development of diseases such as cancer, cystic fibrosis, and sickle cell anaemia.

Microbial genomics has emerged as an indispensable method for the analysis and characterization of many microorganisms, encompassing bacteria, viruses, and fungi. WGS has particularly gained prominence in this field. Through the process of genome sequencing, researchers are able to analyze the genetic makeup of pathogens, enabling them to monitor the transmission patterns of infectious diseases, detect novel variations, and devise efficacious therapeutic interventions.

The utilization of WGS in the domain of agriculture has brought about significant transformations. This technology empowers researchers to raise crop yields, augment nutritional content, and cultivate resistance against pests and illnesses. Through the examination of the genetic compositions of many plant and animal species, researchers possess the ability to discern advantageous genetic characteristics and subsequently incorporate them into novel kinds through selective breeding techniques.

The process of WGS generally encompasses the subsequent stages:

- ✓ The process of sample preparation involves the extraction of DNA from a biological specimen, such as a blood sample, tissue biopsy, or cultured cells.
- ✓ DNA fragmentation is a process wherein the DNA molecule is broken down into smaller fragments, hence enabling easier sequencing
- ✓ The process of library preparation involves the attachment of adaptors to DNA fragments, which facilitates their subsequent attachment to sequencing platforms
- ✓ Sequencing involves the determination of the nucleotide order (A, T, C, and G) in DNA fragments by the utilization of several techniques, including next-generation sequencing (NGS) approaches.

In the field of data analysis, a considerable volume of sequencing data is subjected to computer methodologies in order to effectively compile the genome sequence, detect genetic variations, and elucidate their biological implications. WGS is a technology that is undergoing rapid development, characterized by ongoing advancements in sequencing techniques, tools for data

interpretation, and the cost-effectiveness of genomic sequencing. With the decreasing cost of WGS and the advancement in the interpretation of genomic data, there is a growing anticipation for WGS to assume a progressively significant role in several disciplines, including personalized medicine, evolutionary biology, and agriculture. [25]

1.8 Research Hypothesis:

Pesticides exposure have been found to exhibit mutagenic effects on enteric bacteria, potentially contributing to the rise of AMR. Despite pesticides exposure exhibit mutagenic effects on enteric bacteria, lack of comprehensive review failed to address the scenario properly.

1.9 Research Objective:

This study aims to address the mutagenic effect of pesticides, specifically insecticides, herbicides, and fungicides, on enteric bacteria by conducting a scoping review.

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CHAPTER 2

METHODS:

This study was conducted by following the guidelines and recommendations proposed by Preferred Reporting Items for Systematic Review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR). [26] Overall, this scoping review consisted of five steps suggested by Levac D et al in the year 2010 [27]; (a) identifying the research question, (b) finding relevant studies, (c) study selection, (d) charting the data, (e) collecting, summarizing, and reporting the results.

2.1 Search Strategy and Data Source

A preliminary search was performed to identify the research question and define the study objective. Afterward, discussion with the supervisor has developed the search terms to conduct a thorough search regarding the mutagenic effects of pesticide on enteric bacteria. Key terms used to find appropriate study articles related to our topic of interest are ‘enteric’, ‘bacteria’, ‘enteric bacteria’, ‘enteric pathogen’, ‘pathogenic bacteria’, ‘insecticide’, ‘herbicide’ ‘fungicide’ ‘pesticide’ ‘mutation’, ‘mutagenesis’, ‘mutagenicity’, ‘point mutation’, ‘frameshift mutation’, ‘base-pair mutation’ ‘mutagenic effect’, etc. Details about search terms are available in the supplementary file 4. Four databases (Scopus, and PubMed, Web of Science, Embase) and one search engine (Google scholar) were used as primary data sources for this study.

Between February 2022 and April 2023, an independent search was conducted (after logging out from all Google accounts) to identify pertinent articles published from 1970 to 2022. Subsequently, from July 2022 to August 2023, the findings were meticulously verified and revised the preexisting literature.

Forward and backward search was also conducted manually by checking the citing articles and the reference lists of the individual articles, respectively. To maximize the search efficiency, ‘similar articles’ suggested by the repositories (PubMed, Scopus, etc.) were also explored. Besides to find relevant study articles, going through the corresponding author's profile (Research Gate, ORCID, institutional repositories) has also been done. To verify the specific names of herbicides, the Food and Drug Administration (FDA) pesticide residue monitoring program report for the Fiscal Year 2010 was consulted. [27]

2.2 Eligibility Criteria

The inclusion and exclusion criteria for this study were developed by following the population, context, and concept (PCC) framework developed by Joanna Briggs Institute (JBI) for scoping review. [28]

Articles were included in this review if they fulfilled these criteria – a) studies conducted on bacteria that belong to the Enterobacteriaceae family, b) pesticide exposure resulting in identifiable genotypic and phenotypic changes of the target bacteria, and it is also reported that this change occurs due to mutation, c) involving effects of pesticide mixtures, and also those that highlighted a combination of pesticides with other chemicals, d) original peer-reviewed papers, e) studies written and published in English. In order to have the inclusion as broad as possible, in this study we define mutation as the permanent alterations in the DNA sequence of a cell's genome that are caused by the effect of insecticide. Since genotoxicity involves mutagenicity [29], therefore, the studies that reported genotoxicity rather than mutagenicity was also included here. Furthermore, there were no restrictions on the date of publication and study design (AMES test, WGS), in vitro exposure to pesticides, etc.

The exclusion criteria that were applied in this study included a) studies involving metabolites, derivatives, adjuvants, and analogs of insecticide rather than insecticide its self and their active ingredients. b) articles highlighting other effects than mutagenicity and genotoxicity such as toxicological or cytotoxic effects, c) studies that did not specify the name of the pesticide that caused the mutation, d) review articles, e) letters to editors, f) editorial articles, g) studies written in languages other than English, h) studies that met our inclusion criteria but all the relevant information was not possible to extract without full text.

Mendeley Desktop software (version 2.62.0) was used to manage references and remove duplicates. After removing duplicates, titles and abstracts were screened, after which full-text screening was implemented for the relevant articles. Studies were excluded if the inclusion criteria were not fulfilled, and any discrepancies regarding study selection were resolved through group discussion.

2.3 Data Extraction

As per our inclusion criteria, related papers were included from 1972 to 2023. Data extraction included the following information: references, name of the insecticide, bacteria used, method used to detect mutation, incubation time and temperature, different dose of insecticide that studied to detect mutation, use of metabolic activation, presence of mutation, mutagenic dose, and type of mutation.

Under the guidance of my supervisor, our team independently extracted data in separate Excel files from selected study articles. After that, both of the reviewers cross-checked each other files in order to make the necessary correction. While cross-checking each other files, any disagreement regarding inclusion, exclusion criteria, and result interpretation was resolved through discussion with the supervisor.

CHAPTER 3

RESULTS

In accordance with the guidelines outlined for conducting a scoping review, the process of article selection involves the completion of four distinct processes. The components of identification, screening, eligibility, and inclusion criteria are as follows. By using various databases, including PubMed, Scopus, Google Scholar, Embase, and Web of Science, as the primary sources for the initial search. After applying criteria for removing duplication and other exclusions, a total of 101 articles were selected out of 4069 initial identifications for inclusion in this review. [30-131] The Figure 1 provided has a comprehensive overview of the search technique employed in this study.

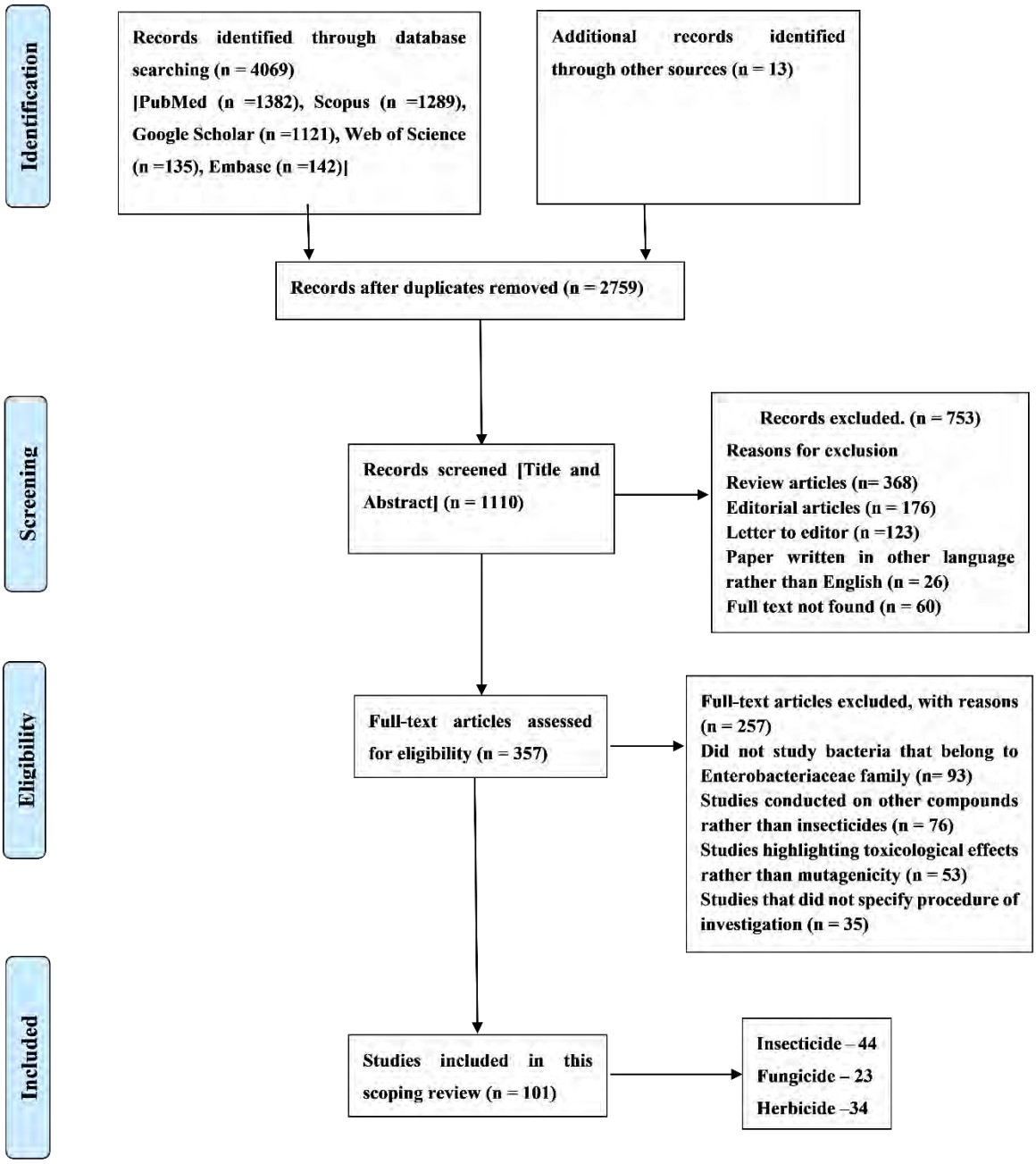

Figure 1 PRISMA chart showing the summary of search result and selection of studies.

Most of the selected study articles was published between 1990-1999 (26 out of 101), following that 1970-1979 (25 out of 101), 1980-1989 (21 out of 101), 2000-2009 (14 out of 101) and 2010-2023 (15 out of 101). To examine mutagenicity, nearly all of the studies used Ames test (98 out of 101), only 3 study used paper disc method, cell microbe coincubation assay and MA/WGS method for assessing mutagenicity of pesticidal compound on Enterobacteriaceae strains. Additionally, *S.* Typhimurium, *E. coli* and *B. subtilis* are the three enteric bacteria we

found to use for mutagenic assessment. Incubation temperature was 37°C and incubation time ranged from 48-72 hours for all most all of the study articles. Frame-shift and base pair mutation are two types of mutation showed in most of studies. Further, out of three different types of pesticides, most of the studies conducted on insecticide (44 out of 101) followed by herbicide (34 out of 101) and fungicide (23 out of 101). Details are available in supplementary file table 1,2,3.

3.1 Insecticides

Our study identified in total 176 insecticides examined for mutagenicity and 60 of them possess the positive result specifically on three organisms – *S.* Typhimurium, *E. coli*, and *B. subtilis*. Among these three organisms, *Salmonella* was reported to use almost all of our study articles. Afterward, *E. coli* reported in 5 studies and *Bacillus* was used only in one study. Moreover, the *Salmonella* strains TA98 and TA100 were the predominant strains utilized in the majority of the selected investigations, accounting for 35 out of 44 and 37 out of 44 studies, respectively. Conversely, the *E. coli* strain Wp2 was reported in only 3 studies, making it the least often employed strain among the selected studies. Moreover, out of 44 studies, 30 studies reported the use of metabolic activation to check mutagenic activity. Among these 30 studies, only two studies mentioned about the use of plant system and rest of study used animal system for metabolic activation (rat liver homogenate).

By following our searching strategy as mentioned earlier, 4069 records identified in total. After removing articles that did not match with our inclusion criteria, 44 articles selected finally for this scoping review**.** See supplementary table 1. Details about the study article selection process is illustrated in figure 2.

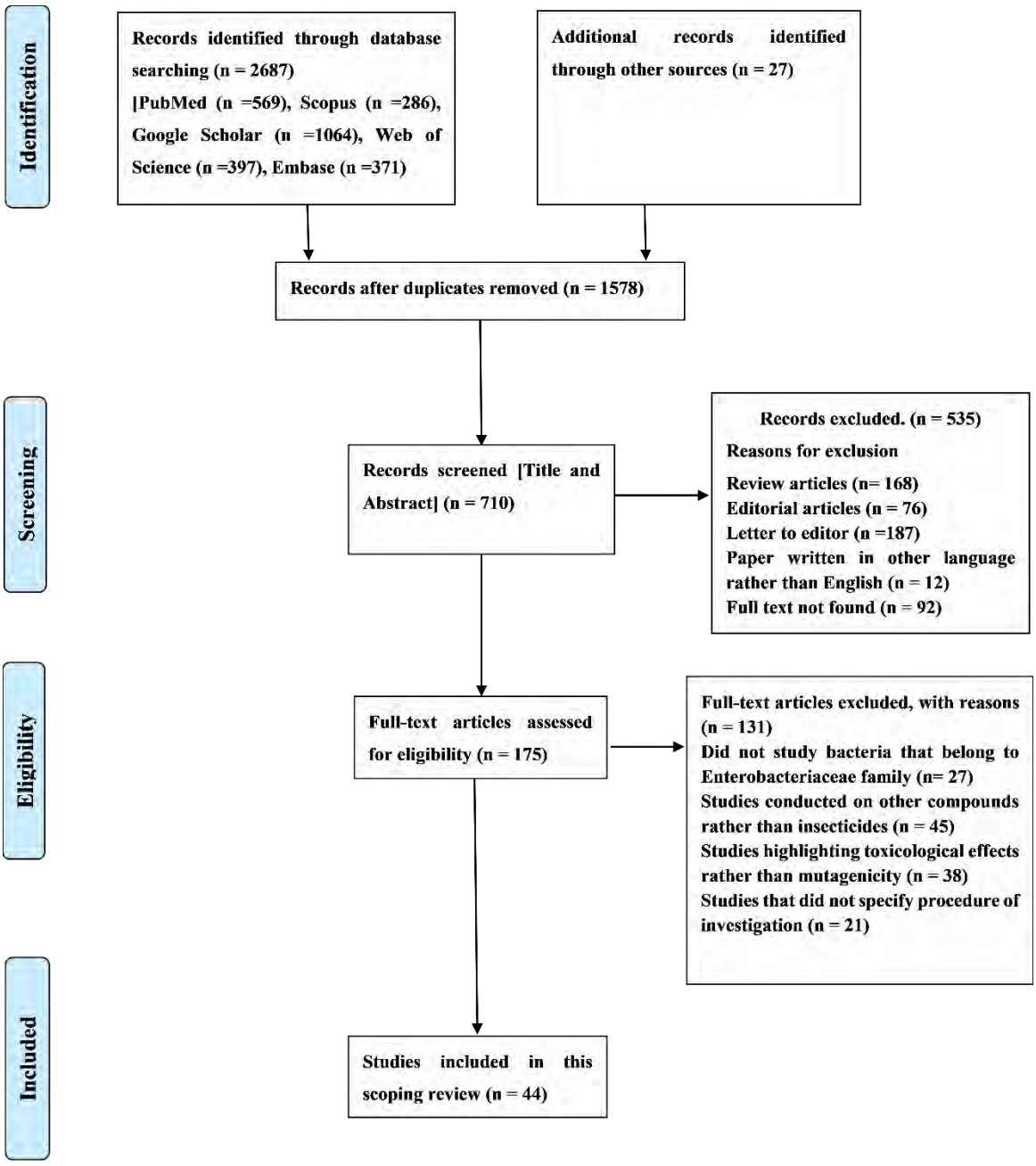

Figure 2 PRISMA chart presenting the summary of search result and selection of studies for insecticides.

3.1.1 Insecticides and Different Hazardous Level

Based on the investigation, it has been shown that insecticides exhibit varying levels of hazard. The study has identified that 44 out of 176 insecticides was categorized as moderately hazardous by World Health Organization (WHO). Following that, 17,10 and 8 insecticidal compounds was categorized as highly, extremely and slightly hazardous respectively. In addition, the report also suggested that only 4 out of 176 insecticidal compounds unlikely to present acute hazard in normal use, whereas of 23 out of 176 pesticides have been deemed outdated or terminated for use as insecticides. Additionally, WHO report did not mention anything about 67 out of 176 insecticides. Details are available in the figure 3 that illustrates the quantities of fungicides and their respective levels of hazard. The topic under consideration is of a significantly hazardous character. The subject matter at hand is characterized by a high degree of peril and danger. The degree of risk is considered to be moderate. The degree of risk is regarded as negligible.

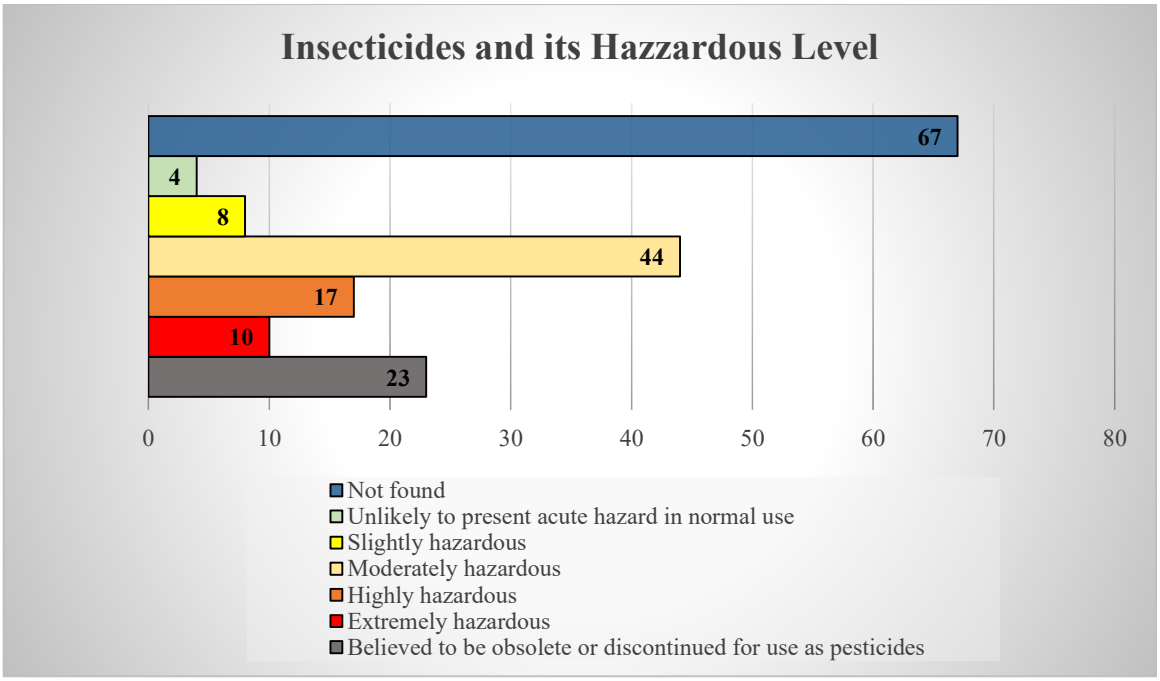

Figure 3 Insecticides and different hazardous level.

3.1.2 Insecticides and Different Categories

We also categorized mutagenic insecticides that are found in human and animal food sample as per the report published by Food and Drug administration agencies (FDA).

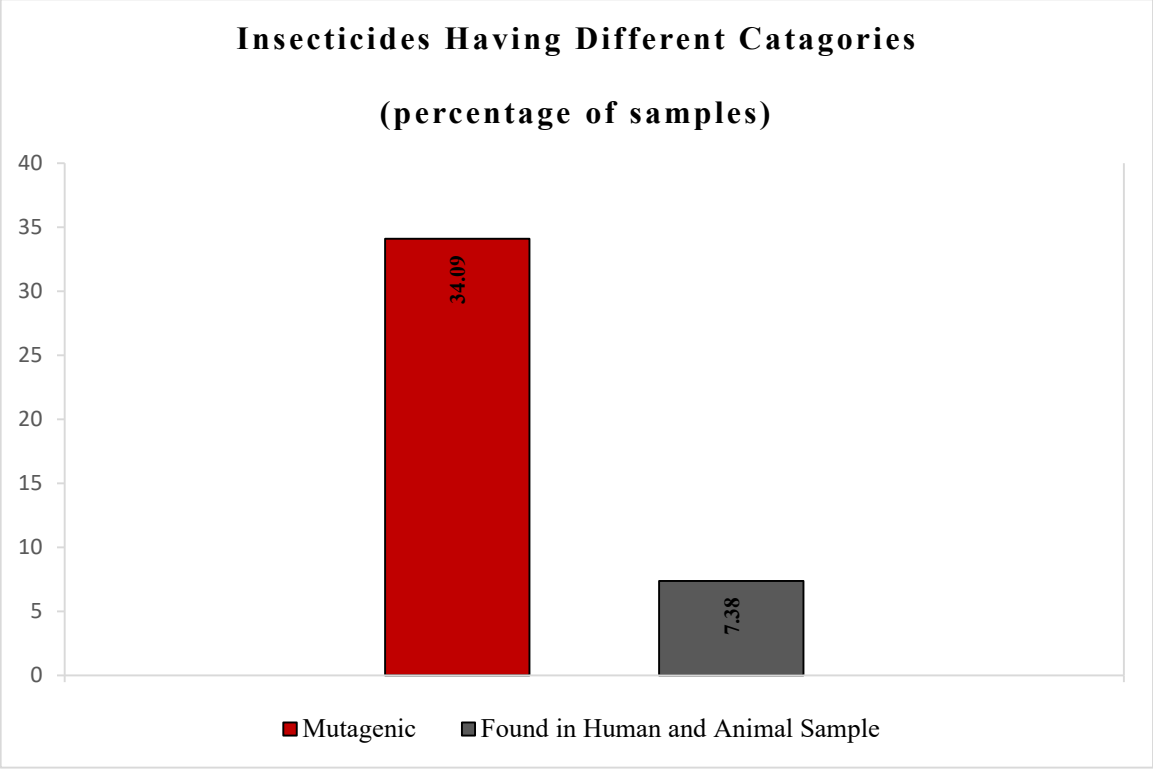

Figure 4 Insecticides and different categories.

The mutagenicity ratio for insecticides (60 out of 176) is depicted in Figure 4, at 34.09%. In contrast, the ratio for human and animal samples (13 out of 176) is 7.38%. This figure provides a succinct representation of the current knowledge regarding the safety implications linked to insecticides. The evidence suggests that specific pesticides have been found in samples collected from both humans and animals. The presented material has the potential to serve as a valuable resource for informing and guiding future research efforts and regulatory actions related to Insecticides.

3.1.3 Mutagenicity of Insecticides on Different Organism

The mutagenic characteristics of 60 pesticides have been assessed in several bacterial species, revealing variability among different organisms. *S.* Typhimurium, was the predominant organism that exhibit 97% mutagenicity (58 out of 60) on different insecticidal compound, whereas *E. coli* 50% ($p = 0.22$). Further, it becomes apparent that their mutagenicity ratio of *B. subtilis* is comparatively much smaller than others.

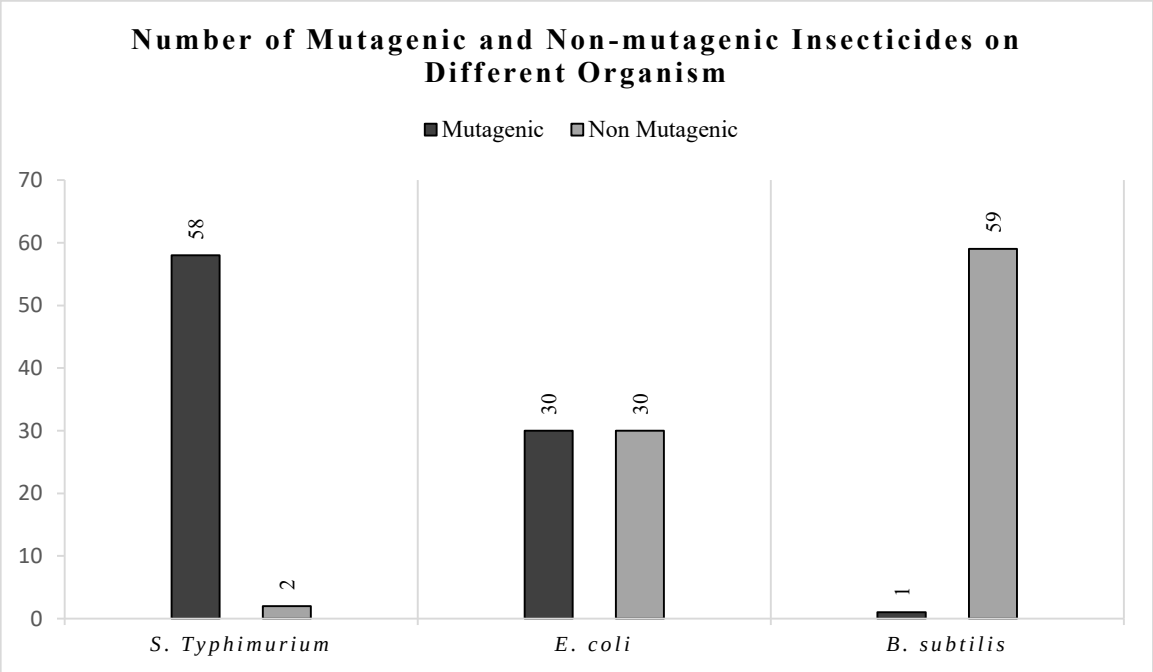

Figure 5 Number of mutagenic and non-mutagenic insecticides on different organism.

3.1.4 Variation of Mutagenic Doses of Insecticides on Different Strains of Same Organism

Based on our comprehensive analysis, a total of 176 insecticidal chemicals have been identified. Out of the total insecticides examined, 60 have exhibited mutagenesis properties on the organisms under investigation. Out of the 60 identified mutagenic compounds, a total of 23 compounds were seen to exhibit mutagenicity at varying doses (referred to as the Mutagenic dosage) across different strains of diverse species. The table 2 provided a comprehensive depiction of the various strains and their corresponding dose.

Table 2 Variation of mutagenic doses of insecticides on different strains of same organism

Name of Insecticide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Acephate	*Salmonella* typhimurium	JK947 Jk3	0.1, 1, 10, and 100 µg/plate	0.1 µg/plate	(Hour, T. C. et al., 1998) [31]
Allethrin	*Salmonella* typhimurium	TA97 TA100 TA104	100, 250, 500, 1000, 1500, 2000, 2500, 3000, and 4000 µg/plate	2000 µg/plate 1500 µg/plate	(Herrera, A. et al., 1988) [32]
		JK947 Jk3	0.1, 1, 10, and 100 µg/plate	0.1 µg/plate	(Hour, T. C. et al., 1998) [31]
Alpha-aminophosphoryl compounds (AP)	*Salmonella* typhimurium	TA100	0.1, 0.5, 1, 5, and 10 mg/ml	0.1mg/ml	(Ilinskaya O et al., 2004) [33]
Azinphos methyl	*Salmonella* typhimurium	TA98 TA100	20, 40, 80, 100, 200 µg/µL	20, 40, 80, 100, 200 µg/µL	(Gómez-arroyo et al., 1987) [36]
Aziphos-methyl	*Salmonella* typhimurium	TA98 TA100	1.3, 1.9, 2.5, 3.8, 4.4, and 5 µg/coincubation	1.3, 1.9, 2.5, 3.8, 4.4, and 5 µg/coincubation	(Gómez-arroyo et al., 2007) [41]
Carbofuran	*Salmonella* typhimurium	JK947	0.1, 1, 10, and 100 µg/plate	0.1 µg/plate	(Hour, T. C. et al., 1998) [31]

Table 2 Variation of mutagenic doses of insecticides on different strains of same organism (continued)

Name of Insecticide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Demond EC 25	*Salmonella* typhimurium	TA98 TA98 TA100	50, 100, 200, 400, and 800 µg/plate	400 and 800 µg/plate	(Shirasu, Y. et al., 1976) [47]
Dibrom (naled)	*Salmonella* typhimurium	TA100 TA1535 TA1535	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al., 1981) [94]
Dichlorvos	*Salmonella* typhimurium	TA1535	Not mention	1.5mg/ml	(Carere, A. et al., 1978) [43]
		TA100 TA100	5, 10, 20, 40, 60 µM/plate	10, 20 µM/plate	(Braun, R. et al.,1982) [44]
		TA1535	Not mention	0.1` ml/plate	(Shirasu, Y. et al., 1976) [47]
Dicrotophos	*Salmonella* typhimurium	TA97a	0.5, 5, 50, 500, and 5000 µg/plate	5000 µg/plate	(Wu J. C.,2010) [113]
Dieldrin	*Salmonella* typhimurium	TA98 TA100 TA1535	1, 25, and 50 µg/ml	25 µg/ml	(Wu J. C.,2010) [113]
Dimethoate	*Salmonella* typhimurium	TA100	0.01, 0.03, 0.1, 0.3, 1.0 and 5.0 mg/well and 0.05, 0.16, 0.5, 1.6 and 5.0 mg/plate	5mg/ plate and 5 mg/well	(Gentile, J. M. et al.,1982) [60]

Table 2 Variation of mutagenic doses of insecticides on different strains of same organism (continued)

Name of Insecticide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Endosulfan	*Salmonella* typhimurium	TA100 TA102	1, 5, 10, and 20 µg/plate	10 µg/plate	(Macgregor, J. T. et al., 1979) [60]
		TA97a TA98	0.25, 0.5, 2, 5, 10, 20, and 30 µg/plate	0.2 µg/plate 2.5 (weak), and 5 µmol/plate	(Olga V. Egorova et al.,2020) [41]
		TA98		5 and 10 µmol/plate (weak)	(Bajpayee, M. et al.,2006)
		TA100		0.5, 1, (weak), 2.5, and 5 µmol/plate	[52]
		TA100		1, 2.5 (weak), 5 and 10 µmol/plate	(Pandey, N. et al.,1990) [53]
		TA98		2.5 (weak), and 5 µmol/plate	
Fonofos	*Salmonella* typhimurium	TA1535 TA1538	Not mention	10µg/plate	(Carere, A. et al., 1978) [43]
Furadan	*Salmonella* typhimurium	TA102 TA104	5, 10, 20 and 30 µg/plate	5 µg/plate	(Saleem U et al.,2014) [56]
Heptachlor	*Salmonella* typhimurium	TA98 TA100 TA1535	Not mention	10µg/plate	(Gentile, J. M. et al.,1982) [54]

Table 2 Variation of mutagenic doses of insecticides on different strains of same organism (continued)

Name of Insecticide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Imidacloprid	*Salmonella* typhimurium	TA98 TA100	25, 50, 75, and 100 µL/plate	25, 50, 75, and 100 µL/plate	(Gentile, J. M. et al.,1982) [54]
Lambda-cyhalothrin	*Salmonella* typhimurium	TA98 TA100 TA100	0.25, 0.5, 1, 2.5, 5, 10, 50 µmol/plate	5, 10 µmol/plate (weak) 10 µmol/plate (weak) 0.5, 1, 2.5, 5, and 10 µmol/plate (weak)	(Karabay, N. U et al., 2005)[62]
Metolcarb	*Salmonella* typhimurium	TA98	0.1, 1, 10, 100 and 1000 µg/plate	0.1, 1, and 10 µg/plate	(Olga V. Egorova et al.,2020) [41]
Monocrotophos	*Salmonella* typhimurium	JK947	0.1, 1, 10, and 100 µg/plate	0.1 µg/plate	(Saleem U et al., 2014) [63]
Phosmet	*Salmonella* typhimurium	TA97 TA100	10, 100, 500, and 1000 µg/dish	62, 185, 556, 1667, and 5000 µg/plate	(Bajpayee, M. et al.,2006) [52]
Phosphamidon	*Salmonella* typhimurium	TA97a TA98 TA100 TA102 TA104	2.5, 5, 7.5, 10 µg/plate	7.5 µg/plate	(Liman, R. et al.,2010) [65] (Hour, T. C. et al.,1998) [68] (Vlckova, V. et al.,1993) [71]

Table 2 Variation of mutagenic doses of insecticides on different strains of same organism (continued)

Name of Insecticide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Phoxim	*Salmonella* typhimurium	TA98 TA100	2.5, 6.3, 9.4, 12.5, 15.6 and 18.8 µg/coincubation	2.5, 6.3, 9.4, 12.5, 15.6 and 18.8 µg/coincubation	(Carere, A. et al.,1978) [76]
Trichlorfon	*Salmonella* typhimurium	TA1535	Not mention	12 mg/ml	(Shirasu, Y. et al.,1976) [108]
Dichlorvos	*Escherichia coli*	B/r try WP2 WP2 try hcr	Not mention	0.1` ml/plate	(Gómez-Arroyo, S et al.,2007) [75]
Dibrom (naled)	*Bacillus subtilis*	TKJ6321	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al.,1981)[97]

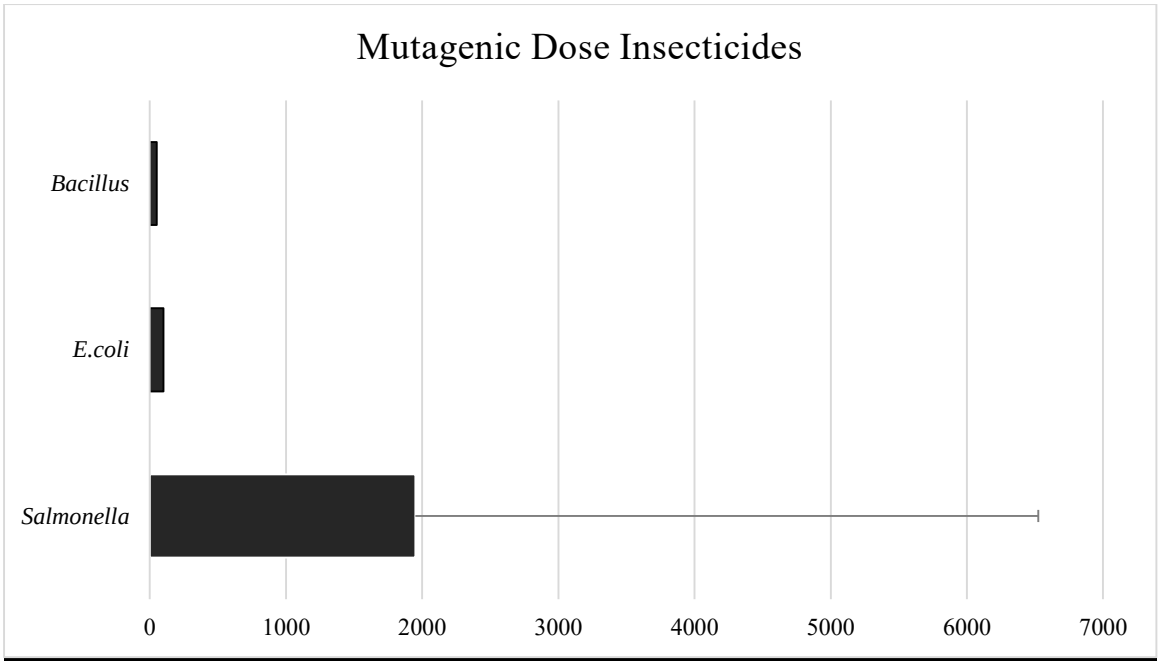

Figure 6 Range of mutagenic doses of insecticides on different organisms.

The mutagenic dose ranges exhibit variability among different strains of *S*. Typhimurium, *E. coli*, and *B*. subtilis, spanning from 100 µg /plate to 5000 µg/plate with mean 1950 µg/plate Standard Deviation 4575 for *S*. Typhimurium. For other two organisms there is no mean and Standard Deviation. This graph (figure 6) provides the graphical representation of mutagenic dose (µg/plate) of insecticides on different organisms.

3.2 Fungicides

Following our searching strategy, as mentioned previously, we were able to identify 1734 records in total. Based on our inclusion criteria, 22 articles were ultimately selected for this scoping review. Details are available in supplementary file 2. Figure 7 depicts details regarding the study articles selection process.

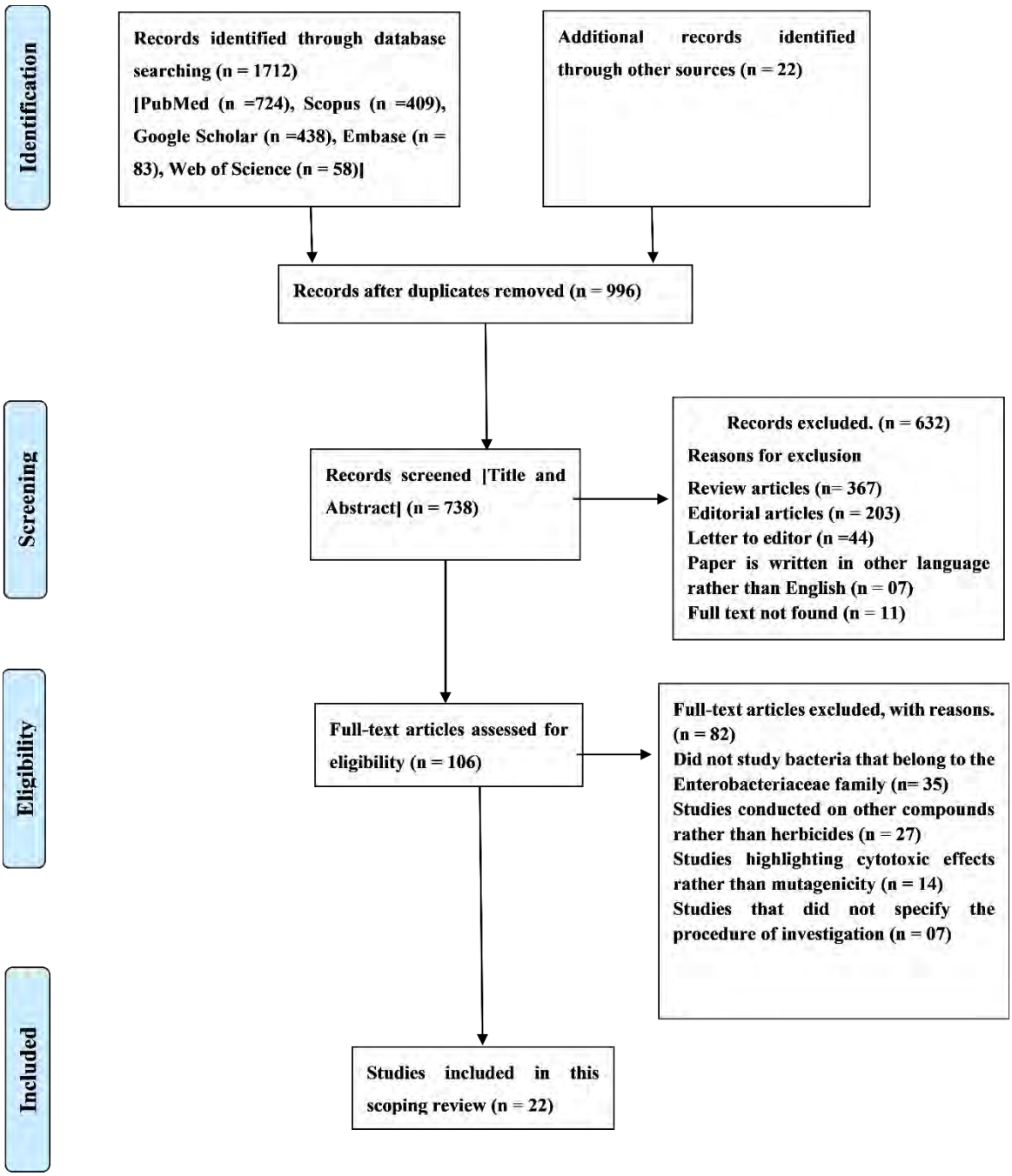

Figure 7 PRISMA chart presenting the summary of search result and selection of studies for fungicides.

Out of the 99 fungicides, 26 showed positive result for mutagenicity, with a notable effect shown against *S.* Typhimurium, *E. coli*, and *B. subtilis*. Among the three species under consideration, it has been observed that a significant majority of our study articles (19 out of 23) have utilized *S.* Typhimurium. Subsequently, three studies have referred to the use of *E. coli*, whereas a solitary study only employed *B. subtilis*. Furthermore, the strains of *Salmonella* TA98 (19 out of 34), TA1535 (17 out of 23), and TA100 (16 out of 23) were the most commonly used strains in most of the studies that were chosen. On the other hand, of the studies that were chosen, the *E. coli* strain Wp2 was reported in only three of them, making it the least frequently used strain. Furthermore, 18 out of 23 studies reporting the using metabolic activation to measure mutagenic activity, with all of these studies employing an animal system for metabolic activation (rat liver homogenate) for metabolic activity.

The present review identifies a comprehensive total of 99 fungicides. The potential mutagenicity of 26 out of 99 fungicides on enteric bacteria such as *S.* Typhimurium, *E. coli,* and *B. subtilis.*

3.2.1 Fungicides and Different Hazardous level

As per our analysis we have found that there are different hazardous levels of fungicides and figure 8 depicts the quantity of fungicides and their corresponding level of hazard. There exist four distinct levels of dangerous classification:

Seventeen of the Ninety-nine fungicides had a WHO classification of moderately hazardous, with an additional 17 fungicidal compound classified as unlikely to pose an acute threat when used normally. Furthermore, 9, 3, and 1 out of 99 fungicides have classified as slightly, highly, and extremely hazardous according to the WHO assessment. On top of that, the report also illustrated that 4 out 99 fungicidal compounds should be obsolete or discontinued for use as pesticide. Additionally, WHO report did not mention anything about 48 out of 99 fungicides.

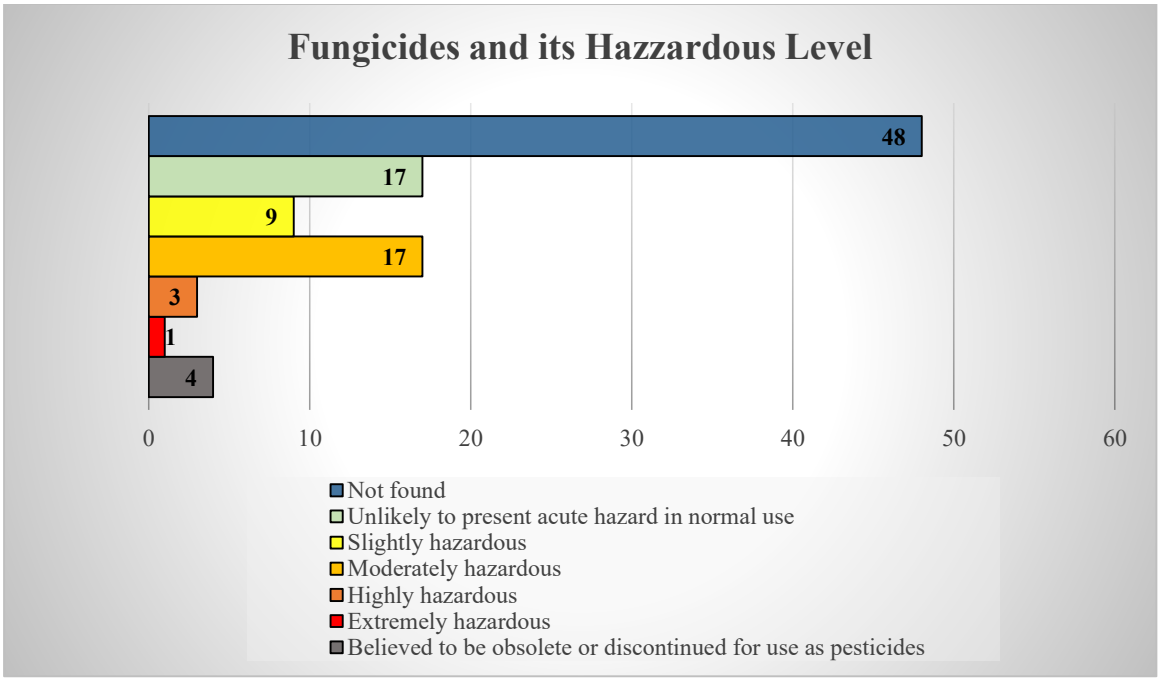

Figure 8 Fungicides and hazardous level.

3.2.2 Fungicides and Different Categories

Another illustration of fungicides has been analyzed in regards of other categories. Figure 9 illustrates the quantitative distribution of fungicides across various categories, as documented in a research study conducted by the Food and Drug Administration (FDA). A total of 99 fungicides were evaluated.

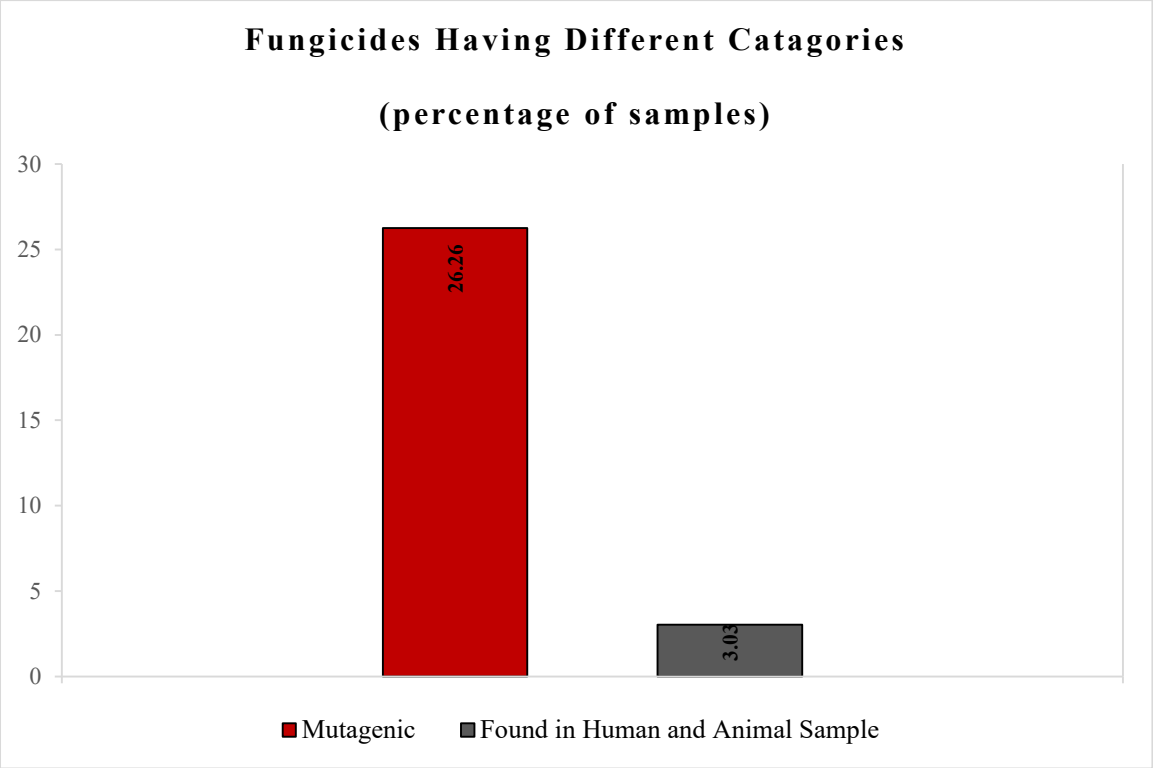

Figure 9 Fungicides and different categories.

Figure 9 shows the mutagenicity ratio (26.26%) for fungicides (26 out of 99). On the other hand, the ratio for samples from humans and animals (3 out of 99) is 3.03%. This graph, is a concise depiction of the present understanding on the safety aspects associated with fungicides. The data indicates that a considerable proportion of fungicides have not undergone comprehensive safety assessments, and that certain fungicides have been detected in samples obtained from both humans and animals. The provided material possesses the potential to inform and direct forthcoming research endeavors and regulatorys measures pertaining to fungicides.

3.2.3 Mutagenicity of Fungicides on Different Organism

The mutagenicity of 26 fungicides has been observed across various bacterial species, exhibiting variability among different organisms. In this case *S.* Typhimurium, was the predominant organism that exhibit 96.15%mutagenicity (25 out of 26) on different insecticidal compound, whereas *E. coli* 34.61% ($p = 0.24$). Further, it becomes apparent that their mutagenicity ratio of *B. subtilis* is comparatively much smaller than others.

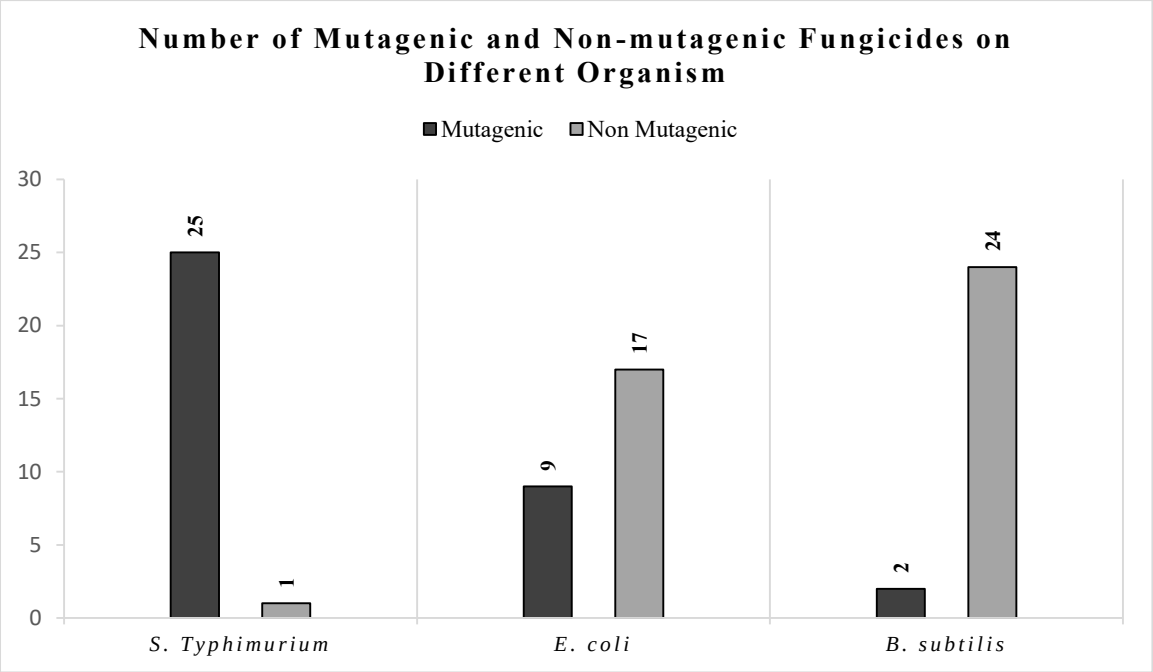

Figure 10 Mutagenicity of fungicides on different organism.

3.2.4 Variation of Mutagenic Doses of Fungicides on Different Strains of Same Organism

Furthermore, it should be noted that the mutagenic dose exhibits variability not just across different organisms, but also among different strains of the same organism. Based on our comprehensive analysis, a total of 99 fungicidal chemicals have been identified. Out of the fungicides examined in our study, a total of 26 have exhibited mutagenesis properties on the organisms under investigation. Out of the 26 identified mutagenic compounds, a total of 11 compounds have been documented with respect to their mutagenic dosage, specifically in relation to various strains of different species. The mutagenic dosage ranges exhibit variability among different strains of various species, spanning from 0.01 µg/plate to 1000 µg/plate. The provided table 3 presents an overview of the mutagenic chemicals and their corresponding doses across various strains.

Table 3 Variation of mutagenic doses of fungicides on different strains of same organism

Name of Fungicide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Thiram	*Salmonella* typhimurium	TA97 TA100 TA102 TA1535	0.01, 0.03, 0.1, 0.3, 1.0 and 5.0 mg/well and 0.05, 0.16, 0.5, 1.6 and 5.0 mg/plate	0.05 and 0.5 mg/plate, 0.01, and 0.1 mg/well	(Olga V. Egorova et al.,2020) [45]
Thiabendazole (TBZ)	*Salmonella* typhimurium	TA98 TA100	5000 - 20000µg	200µg/plate	(Watanabe-Akanuma, M et al.,2003) [104]
NNN	*Salmonella* typhimurium	TA1537	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [59]
NBT	*Salmonella* typhimurium	TA1535 TA1537 TA1538	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [59]
Foltaf	*Salmonella* typhimurium	TA97a	0.5, 1, 1.5, 2, 2.5 µg/plate	1 µg/plate	(Saxena, S. et al.,1997) [99]

Table 3 Variation of mutagenic doses of fungicides on different strains of same organism (continued)

Name of Fungicide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Folpet	*Salmonella* typhimurium	JK947 JK3	0.1, 1, 10, and 100 µg/plate	1 µg/plate	(Hour, T. C. et al.,1998) [42]
		TA100	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al.,1981) [83]
		TA1535	Not mention	100 µg/plate	(Shiau, S. Y. et al.,1981) [83]
Fenoxanil	*Salmonella* typhimurium	TA100	0.1, 1, 10, 100, and 1000 µg/plate	100 and 1000 µg/plate	(Konuk M, 2008) [104]
Dexon (DAPA)	*Salmonella* typhimurium	TA1537 TA1538	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [98]
Carbendazim	*Salmonella* typhimurium	TA97	0.01, 0.03, 0.1, 0.3, 1.0 and 5.0 mg/well and 0.05, 0.16, 0.5, 1.6 and 5.0 mg/plate	5 mg/plate	(Olga V. Egorova et al.,2020) [45]

Table 3 Variation of mutagenic doses of fungicides on different strains of same organism (continued)

Name of Fungicide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Captan	*Salmonella* typhimurium	TA1535 TA97a TA98 TA100 TA102 TA104	20 µg/plate 0.25, 0.5, 1, 1.5 µg/plate	20 µg/plate 1 µg/plate	(Carere, A. et al.,1978) [34]
		JK947 JK3	0.1, 1, 10, and 100 µg/plate	1 µg/plate	(Hour, T. C. et al., 1998) [42]
		TA97 TA98 TA100 TA102 TA1535	0.01, 0.03, 0.1, 0.3, 1.0 and 5.0 mg/well and 0.05, 0.16, 0.5, 1.6 and 5.0 mg/plate	0.05 mg/plate	(Olga V. Egorova et al.,2020) [45]
		TA1535 TA1535	25, 50 µg/plate 10, 25, and 50 µg/plate	25, 50 µg/plate 10, 25, and 50 µg/plate	(Marshall, T. C. et al.,1976) [66]
		TA1537 TA98 TA100 TA102 TA1535 TA1538	25, 50 µg/plate 0.3, 1, 3, 5, 10, 30, 50, µg/plate	25, 50 µg/plate 0.3, 1, 3, 5, 10, 30, 50, µg/plate	(Ruiz, M. J et al.,1997) [112]
		TA98 TA100 TA100 TA1535 TA1535 TA1537 TA1538 TA1538	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al.,1981) [83]

		TA1535	Not mention	50 µg/plate	
Captafol	*Salmonella* typhimurium	TA102	0.1, 0.5, 1, 3, 10, 20, 30, 50, µg/plate	0.5, 1, 3, 5, 10, 20, 30, 50 µg/plate	(Ruiz, M. J et al., 1997)[112]
Thiabendazole (TBZ)	*Escherichia coli*	WP2uvrA	5000 - 20000µg	200µg/plate	(Watanabe-Akanuma, M et al., 2003) [103]
NNN	*Escherichia coli*	B/r try WP2	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [98]
Folpet	*Escherichia coli*	B/r try WP2 WP2 try hcr	Not mention	100 µg/plate	(Shirasu, Y. et al.,1976) [98]
Dexon (DAPA)	*Escherichia coli*	B/r try WP2 WP2 try hcr	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [98]

Table 3 Variation of mutagenic doses of fungicides on different strains of same organism (continued)

Name of Fungicide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
Captan	*Escherichia coli*	B/r try WP2 WP2 try hcr	Not mention	50 µg/plate	(Shirasu, Y. et al.,1976) [98]
Captafol	*Escherichia coli*	B/r try WP2 WP2 try hcr	Not mention	50 µg/plate	
Folpet	*Bacillus subtilis*	TKJ52311 TKJ6321	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al., 1981) [95]
Captan	*Bacillus subtilis*	TKJ5211	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	

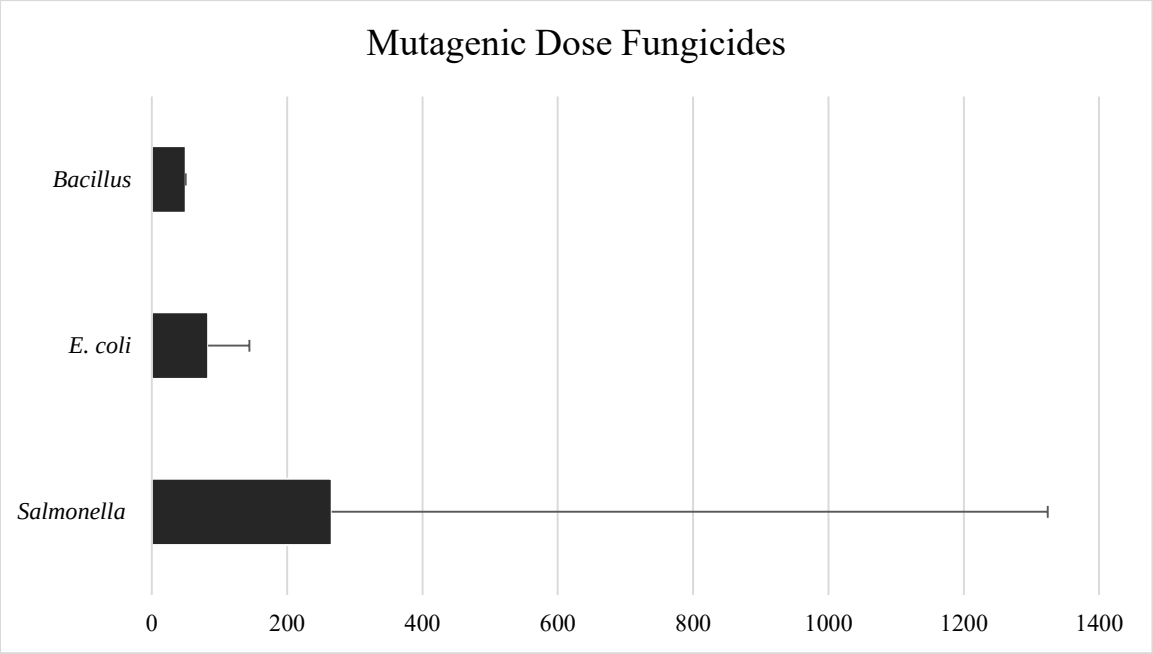

Figure 11 Range of Mutagenic dose of fungicides on different organism

This graph (figure 11) provides the graphical representation of mutagenic dose (µg/plate) of fungicides on different organisms. The average of all mutagenic doses of fungicides for *S.* Typhimurium is 266 and the value of Standard Deviation is 1058 and for *E. coli* the mean is 83 and SD is 61, for *B. subtilis* the value of mean is 50 and SD is 0.

3.3 Herbicides

Our systematic search hit 3008 records in total by following our searching strategy as mentioned earlier. In according to our inclusion criteria, 34 articles selected finally for this scoping review. See supplementary table 3. Details about the study article selection process is showed in figure 12.

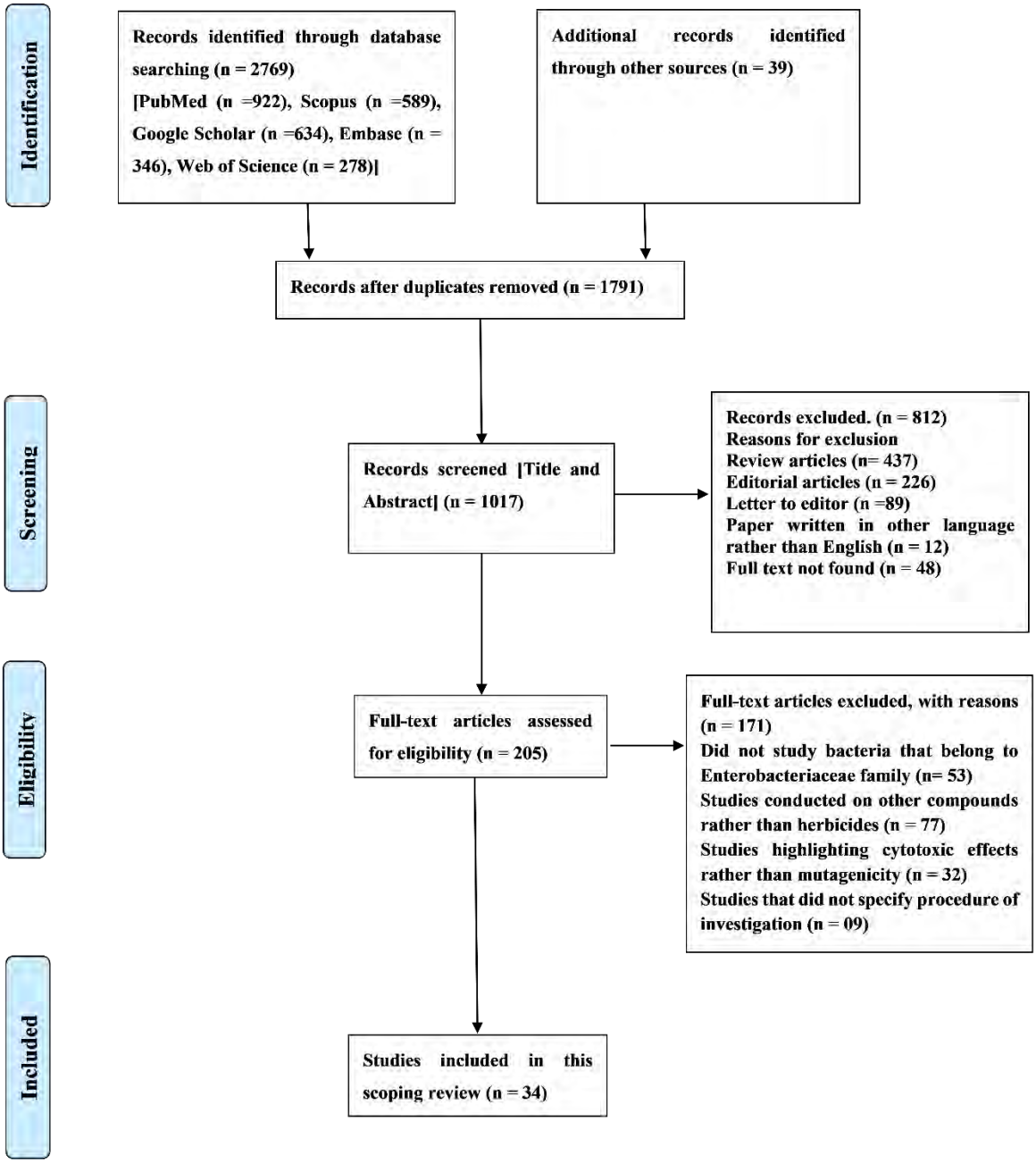

Figure 12 PRISMA chart presenting the summary of search result and selection of studies for herbicides.

Twenty of the One hundred and Ninety-six herbicides that were tested for mutagenicity in our study showed positive results, particularly against S. Typhimurium, *E. coli*, and *B. subtilis*. Among these three species, nearly all of our study articles (32 out of 34) have been reported to use *S.* Typhimurium. After that, 3 studies mention the use of *E. coli* and only single study used *B. subtilis*. Furthermore, the strains of *Salmonella* TA98 (28 out of 34), TA100 (28 out of 34), and TA1535 (27 out of 34) were the most commonly used strains in most of the studies that were chosen. On the other hand, of the studies that were chosen, the *E. coli* strain Wp2 was reported in only three of them, making it the least frequently used strain. Furthermore, 29 out of 34 studies reporting the using metabolic activation to measure mutagenic activity. Only one study used the plant system (Z. mays); the other studies used the animal system (rat liver homogenate) for metabolic activity. The Ames test was mentioned in nearly all of the materials we read as a way to examine the mutagenicity of different bacterial strains. Only a single study mentioned using the MA/WGS method. Metabolic activation was used to inhibit mutagenic activity in 29 of the 34 trials.

3.3.1 Herbicides and Different Hazardous level

The study also revealed that 38 of the 196 herbicides had a WHO classification of moderately hazardous. Furthermore, according to the WHO assessment, 29 out of 196 herbicidal compounds are unlikely to pose an acute threat when used normally, while 18 out of 196 herbicides have had their usage as herbicides judged outmoded or discontinued. The WHO has classified only one herbicide as highly hazardous. Additionally, WHO report did not mention anything about 110 out of 196 herbicides. Supplementary file 3 has more information. Figure 13 illustrates the quantities of herbicides and their respective levels of hazard.

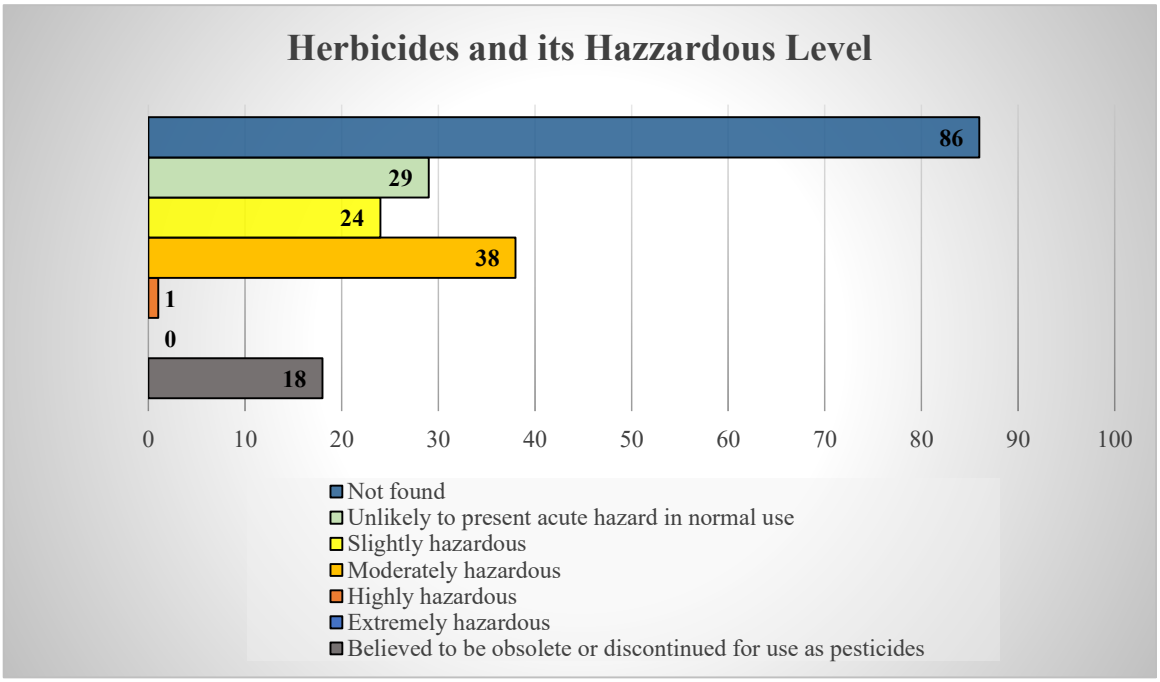

Figure 13 Herbicides and different hazardous level.

The graph depicts a direct relationship between the quantity of herbicides and the level of hazard. This observation suggests that there is a need for additional research and regulatory scrutiny of herbicides.

It is crucial to recognize that the degrees of toxicity linked to herbicides might vary depending on the specific herbicide and its formulation used. Before using any herbicide, it is crucial to conduct a comprehensive examination of the label.

3.3.2 Herbicides and Different categories

The graph provided depicts the quantitative allocation of herbicides among different categories, as reported in a research study undertaken by the Food and Drug Administration (FDA). A comprehensive assessment was conducted on a total of 196 herbicides.

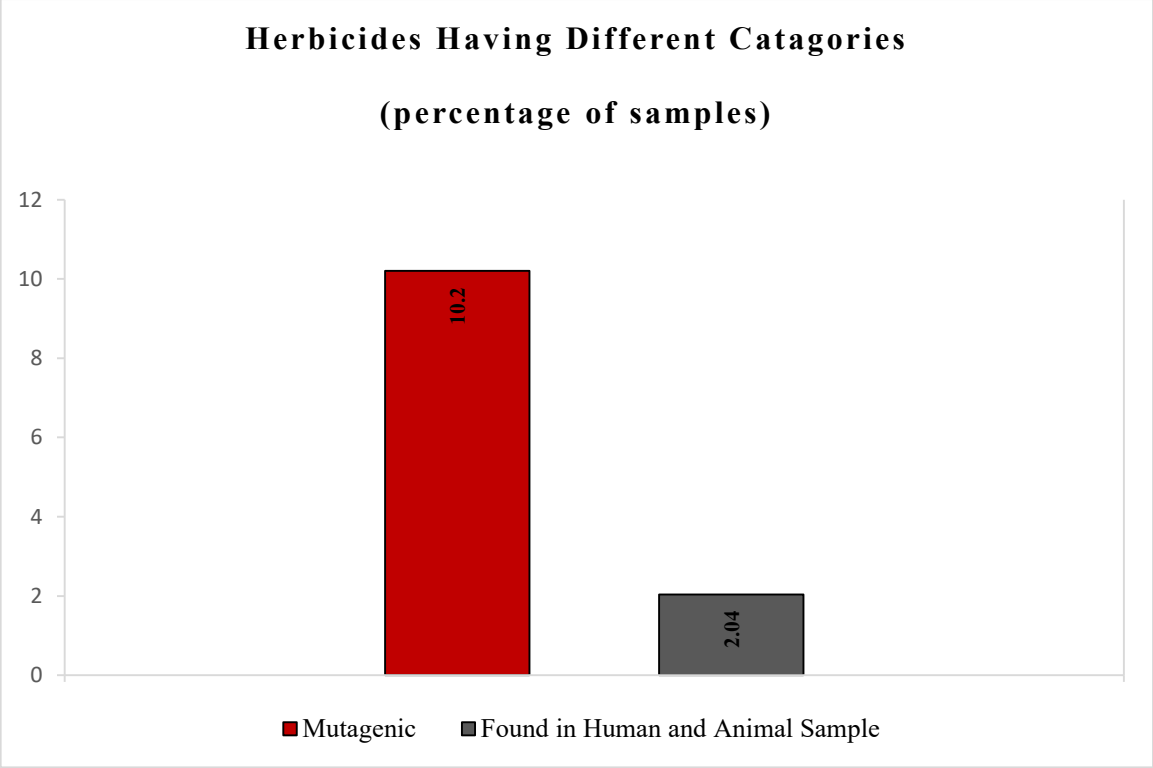

Figure 14 Herbicides and different categories.

Figure 14 shows the mutagenicity ratio (10.20 %) for herbicides (20 out of 196). On the other hand, the ratio for samples from humans and animals (4 out of 196) is 2.04 %. The graph provides a succinct representation of the current knowledge regarding the safety implications linked with herbicides. The available evidence suggests that a significant number of herbicides have not been subjected to thorough safety evaluations, and that specific herbicides have been identified in samples collected from both human and animal subjects. The presented material has the potential to serve as a valuable resource for informing and guiding future research efforts and regulatory actions related to herbicides.

3.3.3 Mutagenicity of Herbicides on Different Organism

Only one of the 20 mutagenic herbicidal compounds demonstrated mutagenicity against *B. subtilis*, and two against *E. coli*. *S.* Typhimurium, was the predominant organism that exhibit 100% mutagenicity (20 out of 20) on different herbicidal compound, whereas *E. coli* shows 10% ($p = 0.21$). Further, it becomes apparent that their mutagenicity ratio of *B. subtilis* is comparatively much smaller than others.

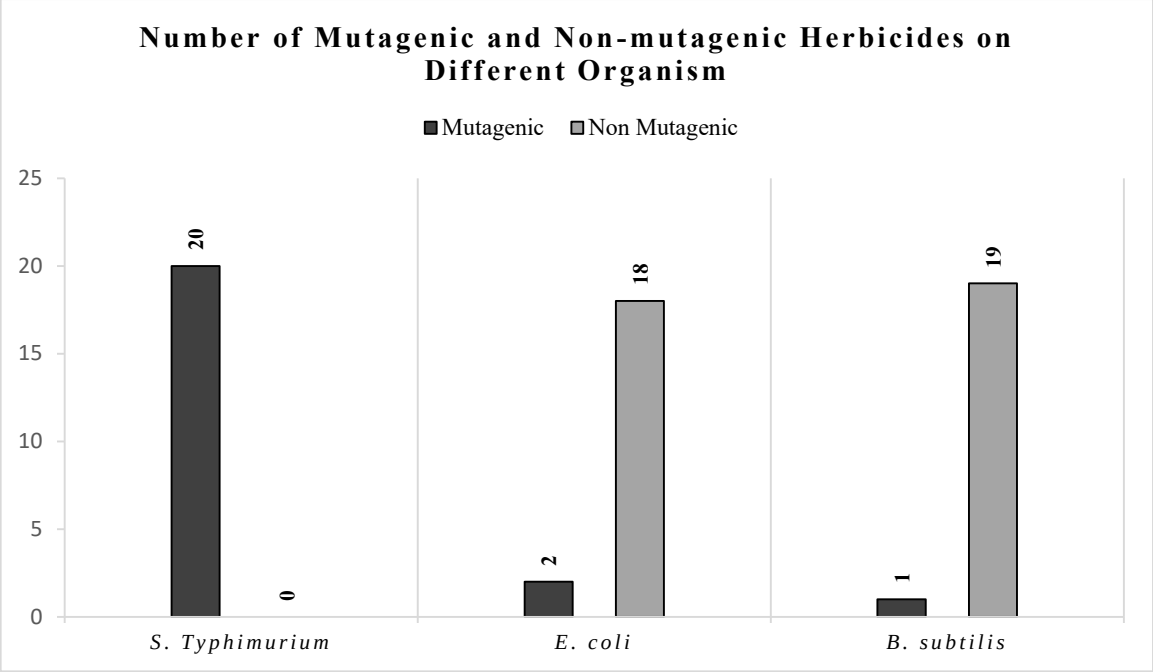

Figure 15 Mutagenicity of herbicides on different organisms.

3.3.4 Variation of Mutagenic Doses of Herbicides on Different Strains of Same Organism

As we know that mutagenic dose has played a crucial role in regards of its mutagenicity. So According to our extensive investigation, a total of 196 compounds with herbicidal properties have been found. Among the entire sample, a specific subgroup of 20 fungicides had mutagenic characteristics when tested on the organisms under scrutiny. Among a collection of 20 substances with mutagenic properties, a subset of 5 compounds has been recognized for their ability to induce mutagenicity at distinct dosages, commonly known as the "Mutagenic dose," in various strains of diverse species. There is considerable variation in the mutagenic dose ranges seen across several strains of varied species, with values ranging from 0.1 milliliters per plate to 4800 micrograms per plate. The table 4 presented herein provides data pertaining to distinct mutagenic agents and their respective dosages.

Table 4 Variation of mutagenic doses on different strains of same organism

Name of Herbicide	Organism	Strain	Dose applied	Mutagenic Dose	Reference
2,4-dichlorophenoxy acetic acid (2, 4-D) 4-chloro-2-methylphenoxyacetic acid (MCPA)	*Salmonella* typhimurium	TA97a TA97a	10, 100, 250, 500, 750, 1000 µg/plate	250, 500 and 750 µg/ plate	(Kappas, A.,1988) [29]
HEH (2-hydrazinoethanol)	*Salmonella* typhimurium	TA1536 TA1537 TA1538	Not mention	0.1ml/plate	(Shirasu, Y. et al.,1976) [38]
Roundup	*Salmonella* typhimurium	TA98 TA100	360, 720, 1081, 1440 µg/plate	360 µg/ plate (Weak) 720 µg/ plate	(Rank, J. et al.,1993) [46]
Triallate	*Salmonella* typhimurium	TA1535	4800 µg/plate	4800 µg/plate	(Carere, A. et al.,1978) [64]
		TA100	50, 100, and 300 µg/plate	100, and 300 µg/plate	(Shiau, S. Y. et al.,1981) [96]
Trifularin	Salmonella typhimurium	TA98 TA100	10, 100, 1000, and 2000 mg/plate	10, 100, 1000, and 2000 mg/plate	(Diril, N. et al.,1994) [78]
HEH (2-hydrazinoethanol)	*E. coli*	B/r try WP2	Not mention	0.1ml/plate	(Shirasu, Y. et al.,1976) [38]
Triallate	*Bacillus subtilis*	TKJ6321	50, 100, and 300 µg/plate	50, 100, and 300 µg/plate	(Shiau, S. Y. et al.,1981) [109]

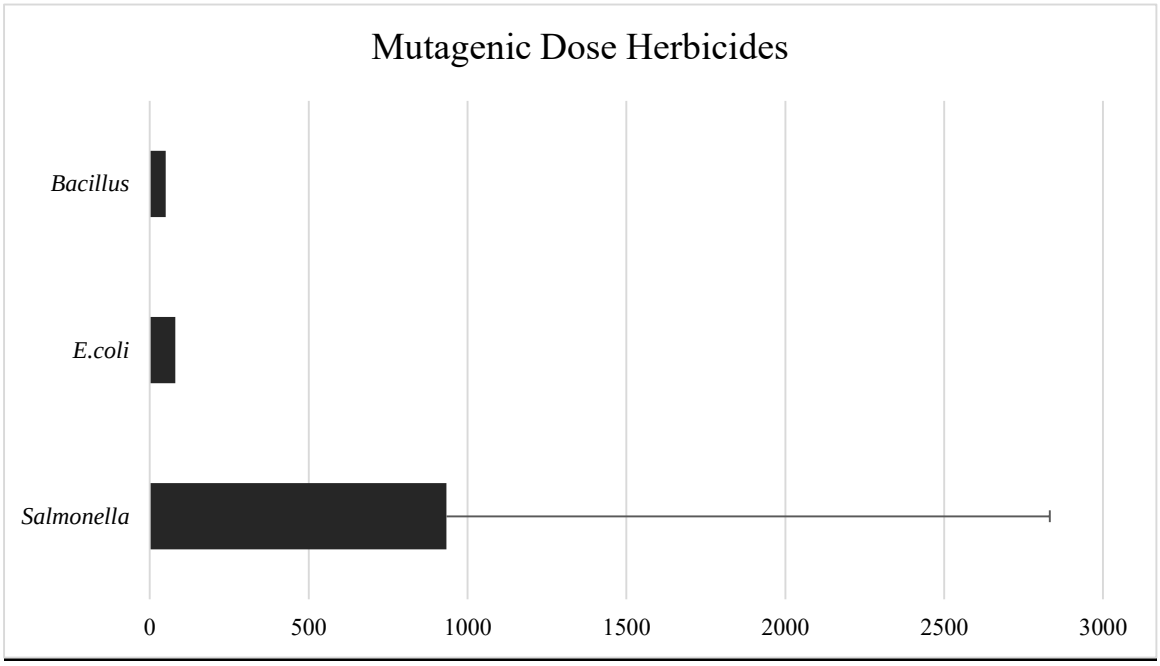

Figure 16 Range of mutagenic doses of herbicides on different organism.

This line graph (figure 16) provides the graphical representation of mutagenic dose (µg/plate) of herbicides on different organisms. This line graph poses a value of 933 as an average mutagenic dose and 1899 as the value of Standard Deviation for *S.* Typhimurium.

3.4 Mutagenic Pesticides and Their Hazardous Level

Findings of the study suggests the relationship between the mutagenic pesticides and their hazardous level mentioned by WHO. Details are available in table 5.

Table 5 Mutagenic pesticides and their hazardous level

Mutagenic Compound	Types of Pesticides	Hazardous Level by WHO
Dieldrin	Insecticide	Believed to be obsolete or discontinued for use as pesticides
Fonofos		
Heptachlor		
Captafol	Fungicide	Extremely hazardous
Phosphamidon	Insecticide	
Dichlorvos		Highly hazardous
Dicrotophos		
Azinphos methyl		
Carbofuran		
Monocrotophos		
Chlorpyrifos		Moderately hazardous
Acephate		
Allethrin		
MCPA	Herbicide	
Dimethoate	Insecticide	
Endosulfan		
Phoxim		
Trichlorfon		
Dibrom (naled)		
Thiram	Fungicide	
Imidacloprid	Insecticide	
Lambda-cyhalothrin		

Metolcarb		
Thiabendazole (TBZ)	Fungicide	Slightly hazardous
Folpet		Unlikely to present acute hazard in normal use
Carbendazim		
Captan		
HEH		Not found
Roundup	Insecticide	
Triallate		
Trifularin	Herbicide	
Alpha-aminophosphoryl compounds	Insecticide	
2, 4-D	Herbicide	
Fenoxanil	Fungicide	
Dexon (DAPA)		
NNN		
NBT		
Foltaf		
Demond EC 25	Insecticide	
Furadan		

3.5 Molecular mechanisms of Mutation find in this study

Frame shift and base pair mutation has been spotted through our study and the molecular mechanism of frame shift and base pair mutation basically depending on some specific factors.

Frameshift mutations occur when certain molecules intercalate between the regular bases, leading to errors in DNA synthesis. Typically, these molecules are flat in shape, like acridine dyes, and possess a hydrophobic characteristic. It is important to note that hydrophobic base stacking plays a role in the formation of the helical structure. A frameshift mutation occurs when one or more additional nucleotides are inserted or deleted. Due to the initiation of the reading frame at the start site, any mRNA resulting from a mutant DNA sequence will be read incorrectly after the insertion or deletion, resulting in the production of a nonfunctional protein. Like a point mutation, a frameshift mutation can result in the creation of a termination codon. Furthermore, frameshift mutations, similar to point mutations, have a reduced negative impact when they occur in proximity to the carboxyl terminus.

Base pair mutations occur when one nucleotide is replaced by another, leading to a modification in the DNA sequence. There are two distinct categories: transitions, which include the substitution of one purine base with another purine base or one pyrimidine base with another pyrimidine base, and transversions, which involve the substitution of a purine base with a pyrimidine base or vice versa.

The effect on the protein is contingent upon the particular amino acid encoded by the modified codon. Occasionally, the replacement can lead to a synonymous mutation, where there is no alteration in the amino acid. Conversely, it can also result in a non-synonymous mutation, which modifies the protein's amino acid sequence. The possible types of mutations are transition mutations and transversion mutations.

3.6 Overall Mutagenicity Ratio in Between the Three Pesticides

Among the overall identified compounds from 101 studies, 23% of them are mutagenic and rest of them are showing non-mutagenic properties. In between the 23% Insecticides shows the highest mutagenicity 57%, following by Fungicide 25% and Herbicide 18%.

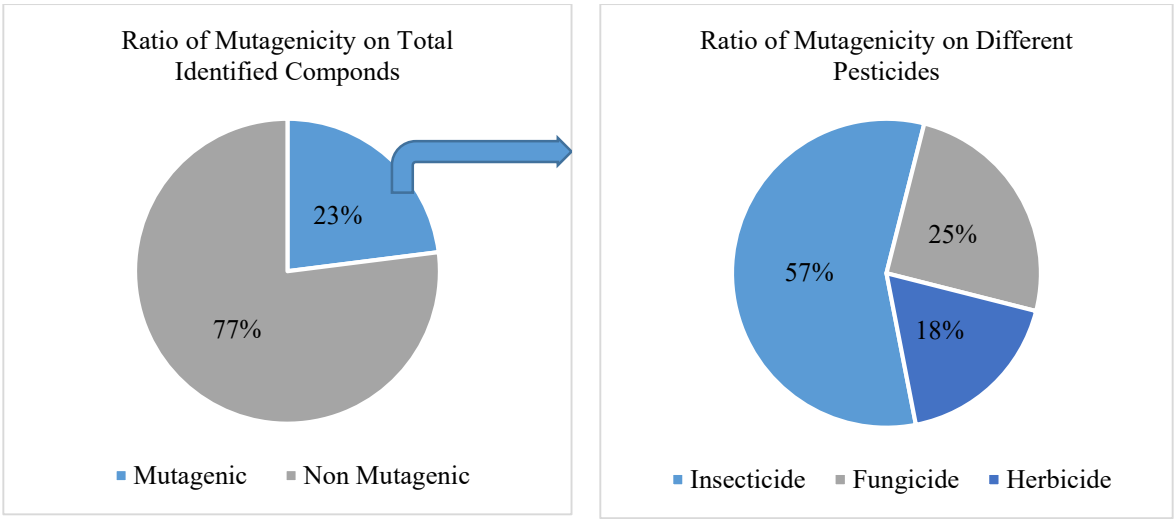

Figure 17 Overall mutagenicity ratio in between the pesticides.

DISCUSSION

This scoping review examines a collection of 101 articles in order to assess the relevance to the hypothesis at hand. The analysis of pesticide-related articles reveals that the majority of publications (26 out of 101), (25 out of 101), (21 out of 101), (14 out of 101) and (15 out of 101) were concentrated within the time frame of 1990-1999, 1970-1979, 1980-1989, 2000-2009, 2010-2023, respectively. This observation suggests a notable surge in research endeavors during the specified decade. In this thesis, we have explored the various perspectives and arguments surrounding the use of pesticides. This concentration of publications within a specific time frame raises questions about the factors that may have influenced this trend. Further exploration is warranted to understand the underlying reasons behind this temporal clustering and its implications for the field of study.

Through in this study, a comprehensive review was conducted to identify the presence of various types of chemicals in agricultural practices. Specifically, the focus was on fungicides, insecticides, and herbicides, which are commonly used in crop protection. The review revealed a total of 99 fungicides, 176 pesticides, and 196 herbicides that are currently being utilized. Upon further analysis, it was found that a significant proportion of these chemicals exhibited potential mutagenicity. Out of the 99 fungicides examined, 26 of them demonstrated mutagenic properties. Similarly, among the 176 pesticides investigated, 60 of them displayed potential mutagenicity. Lastly, out of the 196 herbicides studied, 20 of them exhibited mutagenic effects. These findings highlight the concerning prevalence of mutagenic chemicals in agricultural practices. The presence of such substances raises important questions regarding their potential impact on human health and the environment. Further research and regulatory measures are necessary to address these concerns and ensure the safety of agricultural practices.

In the field of mutagenicity assessment, various bacterial strains such as *S.* Typhimurium, *E. coli*, and *B. subtilis* have been employed. Among these strains, *Salmonella* has emerged as the predominant tester strain in numerous studies investigating the mutagenic effects of pesticides. The predominant use of *Salmonella* strains TA100, TA98, and TA1535, along with the occasional use of *E. coli* strain WP2, was observed in various studies. The Ames test has been widely acknowledged and discussed as a dependable approach for evaluating mutagenicity. In the context of the discussion, it is worth noting that a solitary study made reference to the MA/WGS method. The majority of studies, specifically 18 out of 22, employed metabolic

activation techniques. The study's objective was to investigate and analyze various aspects related to the plant system under investigation.

This study discovered a total of 471 pesticide compounds, of which 106 were found to exhibit mutagenic properties *on S.* Typhimurium*, E. coli,* and *B. subtilis*. The mutagenic dose has exhibited variability across different organisms and strains. Among these, *S.* Typhimurium is the most prevalent bacterium that demonstrates mutagenicity towards insecticides, fungicides, and herbicides. Acephate, Allethrin, Demond EC 25, Dicrotophos, and Lambda-cyhalothrin are examples of insecticidal chemicals that have demonstrated mutagenic properties on several strains of *S.* Typhimurium, but at varied doses with mutagenic effects. Dichlorvos and Dibrom (naled) have been found to exhibit mutagenesis effects on *E. coli* and *B. subtilis*, respectively. Thiram, NBT, Foltaf, Folpet, and Captan are commonly encountered fungicidal agents that have been observed to exhibit mutagenesis effects on *S.* Typhimurium at varying dosages. Interestingly, it has been observed that Captan and Folpet, two types of fungicides, have mutagenesis effects on *S.* Typhimurium*, E. coli,* and *B. subtilis.* These effects vary depending on the dosage and strain of the microorganisms. Triallate has been observed to have mutagenic properties when tested on various strains of *S.* Typhimurium and *B. subtilis*, indicating as an herbicide.

According to the recommendations established by the World Health Organization (WHO), numerous pesticides that have been found exhibit varying levels of hazard, each with its own mutagenic dose. The study conducted has revealed that several insecticides, namely Dieldrin, Fonofos, and Heptachlor, are considered obsolete or have been withdrawn for use as pesticides that also pose mutagenic effects on organisms. Furthermore, the fungicide known as captafol has been widely acknowledged as an extremely toxic substance, exhibiting mutagenesis properties. I have identified other pesticides that exhibit high and moderate levels of hazard according to the criteria established by the World Health Organization (WHO). [132]

One of the most concerning discoveries of the study is the correlation between these mutagenic pesticides and samples of human and animal food. According to a report by the US Food and Drug Administration, pendimethalin, which is classified as a moderately dangerous herbicide, has been detected in samples of both animal and human food. Furthermore, it is worth noting that the food samples include significant amounts of Dichlorvos, Carbofuran, and Monocrotophos, which pose a considerable risk to human health. Heptachlor, an obsolete insecticide, has been detected in human food samples. [133]

Hazardous pesticide poisoning poses a significant global public health concern, resulting in around 300,000 fatalities annually on a global scale. Pesticides are widely employed in both agricultural and domestic contexts. It is widely hypothesized that these substances are responsible for inducing several illnesses in both human beings and wildlife. In recent decades, scholarly investigations have sought to elucidate the underlying mechanisms by which pesticides exert their deleterious effects. Oxidative stress has been identified as a contributing factor in the occurrence of DNA damage, which subsequently increases the risk of developing malignancies and other pathological conditions. The genetic harm induced by pesticides may result from several types of gene alterations, such as insertions, deletions, inversions, and translocations. The genetic elements and/or molecular entities implicated in the modulation of xenobiotic chemical metabolism can be influenced by these mutations. The aforementioned alterations at the genomic level have the potential to generate polymorphisms. This may lead to modifications in the binding affinity between the molecules or potentially influence the level of expression regarding the genes that are influenced by downstream processes. [134] Among the mentioned pesticides some of them are classified as the group of organophosphate. Dichlorvos, phosmet, fenitrothion, chlorpyrifos are some of them. An association between pesticides and AMR has been suggested by some studies. Some strains of pesticide-degrading bacteria isolated from soils exposed to pesticides were resistant to five commonly-used antibiotics. It has been suggested that such resistance could be conferred by a plasmid that contributes to cross resistance via an unspecific organophosphorus hydrolase that also degrades antibiotic derivatives. [135]

This Organophosphorus pesticide (OPPs) is a class of substances frequently encountered in surface water and groundwater coming from agro-industrial processes. The primary degradation of these substances occurs within the interfacial region of cavitation bubbles, mostly due to their hydrophobic and nonvolatile properties. Organophosphates exhibit acute toxicity due to their ability to chemically attach to the acetylcholinesterase enzyme, thereby inhibiting its ability to degrade acetylcholine (ACh). Consequently, insects exposed to organophosphates experience extended muscle contraction and continuous excitement of their nervous system, ultimately resulting in their demise. It is important to highlight that acetylcholine (ACh) serves as a neurotransmitter not just in insects but also in the majority of animal species. Consequently, the potential toxicity of organophosphate pesticides extends to a diverse range of non-target organisms, encompassing people as well. OPPSs chemicals find application as pesticides and insecticides, leading to their accumulation in both soil and aquatic

species. The chemicals in question exhibit structural similarities to chemical warfare agents such as sarin and soman, which exert their effects on the nervous system by covalently blocking the enzyme acetylcholinesterase.

Pesticides with antimicrobial properties such as antibiotics and fungicides are widely used in industrial crop production and could impact AMR in the environment. Antibiotic residues, ARGs and antibiotic-resistant bacteria are released into the environment when manure or sewage sludge are used to fertilize or condition soil, or untreated wastewater is used for irrigation for example, about 11% of all globally irrigated cropland receives inadequately treated wastewater. [136]

Development of antibiotic resistance is a common issue among soil bacteria which are exposing to pesticides continuously at sub-lethal concentration. Soil microorganisms which are being continuously exposed to pesticides develop drug resistance slowly. It has been observed that the multiple antibiotic resistances were increased by the exposure of pesticides. [135]

The indiscriminate utilization of herbicides and pesticides has a significant impact on microbiological processes. The environmental dimension has a crucial role in the emergence and dissemination of antimicrobial resistance (AMR), therefore making it a significant aspect to consider. The genetic change of gut microbiota can be induced by the exposure to agrochemicals, hence potentially contributing to the development of antimicrobial resistance (AMR). [137] A positive association has been seen between the utilization of herbicides and the occurrence of antibiotic resistance. The implications of herbicide exposure on the development of antimicrobial resistance are a cause for concern. The repeated exposure of herbicides during the process of weed management has the potential to induce an enhanced tolerance to herbicides in microorganisms. This phenomenon may exhibit variability both across and within species. [138] The attainment of herbicide tolerance can be accomplished through genetic modifications in the gene targeted by the herbicide [139] or through alterations in non-target genes associated with a broader stress tolerance. [140]

Despite its being a comprehensive review regarding the mutagenic impacts of pesticide compound on enteric Bacteria, it has few limitations as well, one of the major lacking is that the mutagenic dose was not mentioned for all the compounds. Moreover, the study has analyzed the free access articles only. Paid articles may have provided us with more

information The results are not statistically conclusive as most of the test was performed for *S.* Typhimurium and in case of *E. coli* and *B. subtilis* the data was not available. Another important limitation of the study is that the relation towards dissemination of AMR and hazardous level has not been clearly defined.

It's clear that we can't live without medicines or pesticides, but we need to keep a balance, which means we will have to use these things more and more carefully. It can be harder to treat a sickness if it is resistant to even one drug. We should try to stop or slow the growth of antimicrobial resistance whenever we can. Risk assessment of uncontrolled use of pesticides on different environmental conditions should be analyzed and depending on the results regulations on pesticide usage on specific doses should be implemented. Collaborative research with the government agencies and other experts from private sector can come up with an impactful outcome regarding this. This will help keep you from getting infections and make antibacterial drugs work longer. We need to learn more about the role of pesticides as both toxins and ways for AMR to spread. This will help solve the problem of antibiotic resistance and make more people aware of the need to regulate and watch the environment.

CONCLUSION

The significance of pesticides in relation to the development of antimicrobial resistance (AMR) is cause for concern. It is crucial to gather additional evidence regarding the impact of pesticides on bacterial antibiotic susceptibility and the generation of temporary adaptive responses.

AMR can be developed within the natural environment through the contamination of ecosystems by antibiotics and agrochemicals used in both clinical and agricultural practices. This contamination leads to alterations in the structure of bacterial communities and the presence of environmental resisters. The multifaceted impacts of pesticide use, along with the potential synergistic effects resulting from the combination of pesticides and other developing pollutants, can lead to the development of cross-resistance and necessitate careful consideration.

AMR can manifest both in the presence and absence of antibiotic molecules, and may emerge in geographically distant areas, apart from clinical settings. The transmission of antibiotic resistance genes (ARGs) from ambient bacteria to pathogenic bacteria is a crucial factor in the development and spread of antibiotic resistance. The assessment of soils in flood plains of

polluted rivers holds significant importance in identifying the sources of antimicrobial resistance (AMR) and understanding the effects of pesticides on the transmission of resistant genes.

The escalation of pesticide use in agricultural practices is undeniably accountable for the selection and subsequent establishment of phenotypes in pathogenic strains that exhibit resistance to various antibiotics. After isolates are exposed to antibiotics and other chemical agents that may exert a selective pressure, an altered phenotype results. The excessive utilization of antimicrobials may result in an elevated likelihood of spontaneous mutations, ultimately resulting in heightened levels of resistance that will compromise the efficacy of antibiotic treatment.

SUPPLEMENTARY DETAILS

https://drive.google.com/drive/folders/1IgDEIJuFPmQm5ZhLWMBG0i-8CcUL9btp

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