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ASSESSMENT OF LIVER FUNCTION TESTS, RENAL FUNCTION TESTS, SERUM CHOLINESTERASE LEVEL, KNOWLEDGE, ATTITUDE AND PRACTICE AMONG HORTICULTURE WORKERS AT TANA FLORA FLORICULTURE INDUSTRY, BAHIRDAR, ETHIOPIA, 2025: A COMPARATIVE CROSS-SECTIONAL STUDY.

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This is to certify that the thesis prepared by Habtamu Molla Gietie, entitled **Assessment of liver function tests, renal function tests, serum cholinesterase level, knowledge, attitude and practice among horticulture workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025: a comparative cross-sectional study.** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations and Acronyms

ACHE	**Acetylcholine esterase**
AD	Alzimer disease
A/G ratio	Albumin to globulin ratio
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
API	Acute pesticide intoxication
AST	Aspartate aminotransferase
BchE	Butyrylcholinesterase
BUN	Blood urea nitrogen
CKD- EPI	Chronic Kidney Disease Epidemiology Collaboration
CM	Carbamates
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
DBL	Direct Bilirubin
eGFR	Estimated glomerular filtration rate
EPA	Environmental Protection Agency
EPHI	Ethiopian Public Health Institute
EU	European Union
GIS	Geographical information system
GLP	Good laboratory practice
IFCC	International Federation of Clinical Chemistry
IQC	Internal quality control
LD50	Lethal dose 50
KAP	Knowledge, Attitude and Practice
LDH	Lactate dehydrogenase
LFTs	Liver function test
OP	Organophosphate
PPE	Personal protective equipment
RFTs	Renal Function tests
SOP	Standard operating procedure
SPSS	Software package for social science
SST	Serum separator tube
TPRI	Tropical Pesticide Research Institute
UAPP	Unintentional Acute Pesticide Poisoning
UNFAO	United Nations Food and Agriculture Organization
USD	United States dollar

Abstract

Background: Workers in the floriculture industry are exposed to several pesticide classes through various routes, including skin contact, inhalation, and ingestion. Although many pesticides are known to be hepatotoxic and nephrotoxic, there is no published article on the effect of pesticides on the liver and renal in Ethiopia, particularly in the Amhara region.

Objective: This study aims to Asses liver and renal function tests among Horticulture workers at Tana flora floriculture, Bahirdar, Ethiopia, 2025.

Methods: A comparative cross-sectional study was conducted from February 8 to May 28, 2025, involving 51 floriculture workers and 51 residents of Zege town as controls. Socio-demographic, clinical, and behavioral data were collected using structured questionnaires. 5 milliliters of venous blood were drawn to measure RFTs, LFTs, and BChE levels on a Cobas 6000 analyzer. Data entry was done with Epidata 4.7 and analyzed using SPSS version 27. Descriptive findings were presented with tables and graphs, while chi-square/Fisher exact test, t-tests, and Kruskual-Wallis test were used for comparisons. Stepwise multiple linear regression identified key determinants, with significance set at $p<0.05$.

Result: Floriculture workers had significantly higher mean ALT, AST, DBL, globulin, urea, creatinine, and BUN/creatinine ratio, but lower albumin, A/G ratio, BChE, and eGFR than controls ($p<0.05$). Workers with >10 years of exposure showed elevated ALT, AST, TBL, DBL, globulin, urea, and creatinine, and reduced albumin, A/G ratio, and eGFR ($p<0.05$) compared to those with <5 years of exposure. Increased work duration was significantly associated with increased ALT, AST, TBL, and globulin, but negatively with the A/G ratio. Increased age was significantly associated with increased creatinine and decreased eGFR, while higher BMI was associated with increased urea and creatinine. Regular PPE use ($\beta = 724.28$, $p = 0.005$) and periodic health checkups ($\beta = 936.03$, $p = 0.020$) are associated with increased BChE, whereas female sex ($\beta = –697.99$, $p = 0.009$) and higher BMI ($\beta = –203.64$, $p = 0.002$) are associated with low BChE. Overall, 51% of workers had good knowledge, 51% had a positive attitude, and 66.7% had good practices toward pesticide exposure.

Conclusion: Our study found that floriculture workers had altered liver function tests, renal function tests, and BChE levels than controls, associated with pesticide exposure in the floriculture industry. It depends on the time of exposure to pesticides in the floriculture industry. Prolonged exposure to pesticides leads to more alterations of liver function tests, renal function tests. Utilization of personal protective equipment in work area is essential to maintain normal BChE level

Keywords: Floriculture, Horticulture, Liver function, renal function, Choline esterase

1. Introduction

1.1. Background

The term "horticulture" comes from the Latin words "hortus" and "cultura," which mean "garden" and "cultivation," respectively (1). Horticulture has been divided into three major groups: pomology, olericulture, and floriculture. Pomology is the production of fruit crops, encompassing their cultivation, picking, and management after harvest (2). The area of horticulture that deals with growing vegetables is called olericulture. The term "floriculture" refers to the branch of horticulture that focuses on growing decorative and flowering plants for gardens and floristry, which together make up the floral business (3).

Floriculture is one of the rapidly evolving agro-industrial sectors in Ethiopia. Over the past two decades, the industry has expanded significantly, contributing to employment creation, export diversification, and foreign exchange earnings. The sector provides job opportunities for tens of thousands of people, with women constituting the majority of the workforce, thereby playing an important role in income generation and poverty reduction. In economic terms, floriculture has become a major source of foreign exchange; for instance, Ethiopia earned approximately USD 212 million from cut-flower exports in 2017, underscoring the sector’s growing contribution to the national economy (4). Despite its substantial economic benefits, the health implications of the floriculture sector remain insufficiently investigated. Pesticides are widely applied to enhance productivity, yet chronic occupational exposure may result in adverse health outcomes among workers.

Pesticides are chemical substances used to kill, control, or repel pests, and they are widely applied in agriculture to enhance crop protection and improve productivity (5). Workers in horticulture often absorb pesticides by ingestion, inhalation, or skin contact, which are then transported through the circulatory system to impact different organs. It is widely known that the detoxification of xenobiotics is primarily dependent on the liver and kidneys and related compounds through metabolism and excretion. Hence, xenobiotics such as pesticides have the capacity to severely damage both organs (6).

The liver is a key organ in the detoxification process because of the problem of maximum exposure to xenobiotics and their metabolic byproducts (7, 8). The liver is the principal site of xenobiotic metabolism and is crucial for maintaining homeostasis. However, phase I microsomal oxidation processes cause many chemical agents to undergo bioactivation rather than detoxification. This produces hazardous metabolites and reactive intermediate species that can cause liver injury. Hepatocellular necrosis may result from such hazardous metabolites' interactions with intracellular macromolecules. Nonetheless, the metabolism and toxicity of pesticides and their metabolites may be affected by genetic variations in the genes encoding phase I xenobiotic metabolizing enzymes (9).

Phase I xenobiotic metabolism entails hydrolytic, reductive, and oxidative processes that expose or add functional groups to lipophilic substances, boosting their chemical reactivity and enabling conjugation events later on. The cytochrome P450 (CYP) superfamily, which is essential for the metabolism of pesticides and other environmental toxins, primarily catalyzes these biotransformation pathways (10, 11).

The heme-containing monooxygenases known as cytochrome P450 enzymes are mainly found in the smooth endoplasmic reticulum of hepatocytes, while they are also expressed in extrahepatic organs such the kidney, lung, intestine, and brain. NADPH–cytochrome P450 reductase provides the molecular oxygen and reducing equivalents needed for CYP-mediated processes, which insert one oxygen atom into the substrate while reducing the other to water. CYP enzymes use these processes to change parent pesticides into more polar metabolites that can be further bio activated or detoxified (10, 12).

Significant inter individual variation in enzyme expression and catalytic activity is caused by genetic polymorphisms in genes encoding Phase I oxidase enzymes, specifically CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Even under similar exposure settings, these genetic variations categorize people as poor, intermediate, extensive, or ultra-rapid metabolizers, changing internal pesticide dose, metabolite production, and susceptibility to harmful health effects (11).

Changes in CYP-mediated metabolism have significant toxicological implications since increased activity may result in the production of reactive or hazardous intermediates, while decreased activity may cause the buildup of parent chemicals. These pathways highlight the importance of gene–environment interactions in occupational health risk assessment by contributing to differential vulnerability to pesticide-induced hepatotoxicity, nephrotoxicity, oxidative stress, and cholinesterase inhibition (13).

Liver function tests (LFTs) are frequently used screening blood tests for abnormality of liver. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total protein, total bilirubin (TBL), direct bilirubin (DBL), gamma-glutamyltransferase (GGT), and others are among them. These enzymes and proteins change in the blood when liver cells are injured. ALT and AST mainly reflect hepatocellular damage, while ALP is most useful for detecting bile duct disorders. Elevated levels can signal inflammation or liver dysfunction. Pesticides, including insecticides, herbicides, and fungicides, are well known to be hepatotoxic (14-16).

The kidney, with its high blood flow and toxin-concentrating tubular system, is highly vulnerable to xenobiotic toxicity. However, early detection of renal injury remains challenging (9). The kidneys regulate fluid balance and remove waste from the blood. Renal function tests (RFTs), including urea, uric acid, creatinine, estimated glomerular filtration rate (eGFR), and electrolytes, are used to assess how well the kidneys are working (17, 18).

Blood cholinesterase testing is widely used to monitor exposure to carbamates (CM) and organophosphorus (OPs) pesticides. Red blood cells and brain tissue contain true acetylcholinesterase (AChE) (EC 3.1.1.7), whereas the liver produces butyrylcholinesterase (BChE) (EC 3.1.1.8), which is present in serum. Pesticide exposure is strongly linked to reduced cholinesterase levels (19). Many OP and CM compounds exert their acute toxicity through inhibition of AChE (20).

Organophosphorus esters inhibit AChE by phosphorylating the serine hydroxyl group at its active site. Unlike the normal acetylated form, which quickly breaks down to regenerate the enzyme, the phosphorylated form is highly stable. In some cases, depending on the phosphorus substituents (R and R’), inhibition becomes irreversible (21). CM insecticides inhibit AChE

through carbamylation of the serine hydroxyl group after forming an enzyme inhibitor complex. This blocks enzyme activity similarly to organophosphorus esters. However, unlike them, the carbamylated enzyme can spontaneously regenerate, releasing methylamine and carbon dioxide (22).

1.2. Statement of problem

Since beginning in the late 1990s, Ethiopia's floriculture sector has grown to become Africa's second-largest exporter of flowers to the European Union (EU), next to Kenya. The sector has greatly increased the nation's foreign exchange profits by providing employment opportunities for people with low levels of education and those living in poverty (15).

Over 4 million tonnes of active ingredients are used in commercial mixed pesticides annually. From 1993 to 2016, less than 170 nations routinely reported national statistics to the United Nations Food and Agriculture Organization (UNFAO); therefore, this number, which equates to about 0.5 kilograms per person year, likely underestimates the real worldwide consumption (23).

The World Health Organization (WHO) reports that there are one to five million incidents of poisoning among agricultural workers each year, with varied degrees of severity and the potential for damage to vital organs like the heart, kidneys, or lungs. Unintentional pesticide poisoning (UAPP) has been estimated to be the cause of 11,000 deaths worldwide each year. South and South-Eastern Asia had the highest number of UAPP cases, followed by East Africa, which includes Ethiopia (24, 25).

Plants, animals, people, and the abiotic component are all severely harmed by pesticide use (26). The hepatotoxic and nephrotoxic effects of agricultural pesticides have been shown in several laboratory animal studies (27, 28). There have been reports of farmers from Tunisia and India experiencing altered liver and renal function (29, 30). Additional studies also found a favorable correlation between liver dysfunction indicators and pesticide exposure (31, 32).

Exposure to organophosphorus pesticides (OPs) in a work setting or environment can have harmful effects on human health. Only around 0.1% of the pesticides that are administered really reach the intended pests; the remainder is dispersed through food, soil, and water (33).

They primarily damage the exposed organism's nervous systems by increasing acetylcholine levels in the cholinergic synapse and inhibiting acetylcholinesterase (AChE). Besides cholinergic effects, OPs induce oxidative stress (34, 35). Affect metabolic pathways (36), and cause multiple types of organ dysfunctions, including hypoxia and insufficient tissue perfusion of the heart and liver (37). They produce ultra-structural, biochemical, metabolic, and mitochondrial damage in the liver, which is demonstrated by alterations in hepatic biomarkers such serum aminotransferase, DBL, and TBL (38-40).

The fast expansion of flower farms in Ethiopia has led to an increase in pesticide use in the country's agricultural sectors in recent years (41). Between 2002 and 2006, 13,381 tons of pesticides were imported; between 2006 and 2011, this amount increased to 30,059 tons (42). On flower farms, OP and CMs are frequently used pesticides (43). The measurement of cholinesterase levels in workers' blood is an useful indicator of human exposure to OPs and CMs (44).

Workers at Ethiopian greenhouse farms that grow cut flowers have reported a range of health problems, including foot edema and kidney problems. High frequencies of cutaneous, respiratory, and abnormal serum cholinesterase levels have been reported (45, 46). Ethiopia, self-reported acute pesticide intoxications (API) among large flower farm employees revealed a 26% overall acute pesticide intoxication rate. Nervous system symptoms were the most common, followed by cardiovascular, gastrointestinal, respiratory, ophthalmic, and dermatologic factors (47).

Although several studies worldwide have reported the adverse effects of occupational pesticide exposure on liver and renal function profiles, to the best of the principal investigator’s knowledge, no similar study has been published in Ethiopia in general, particularly study area. Therefore, this study was aimed to assess the effect of pesticide exposure on liver using LFTs

(ALT, AST, ALP, total protein, Albumin, DBL, TBL, and Albumin to globulin (A/G) ratio and kidney using RFTs (urea, creatinine, and uric acid, eGFR, BUN to creatinine ratio) and BChE in floriculture industry workers. To address this gap, this research was done to evaluate the liver and renal function of horticultural workers and identify potential risk factors associated with their occupational environment.

1.3. Significance of the study

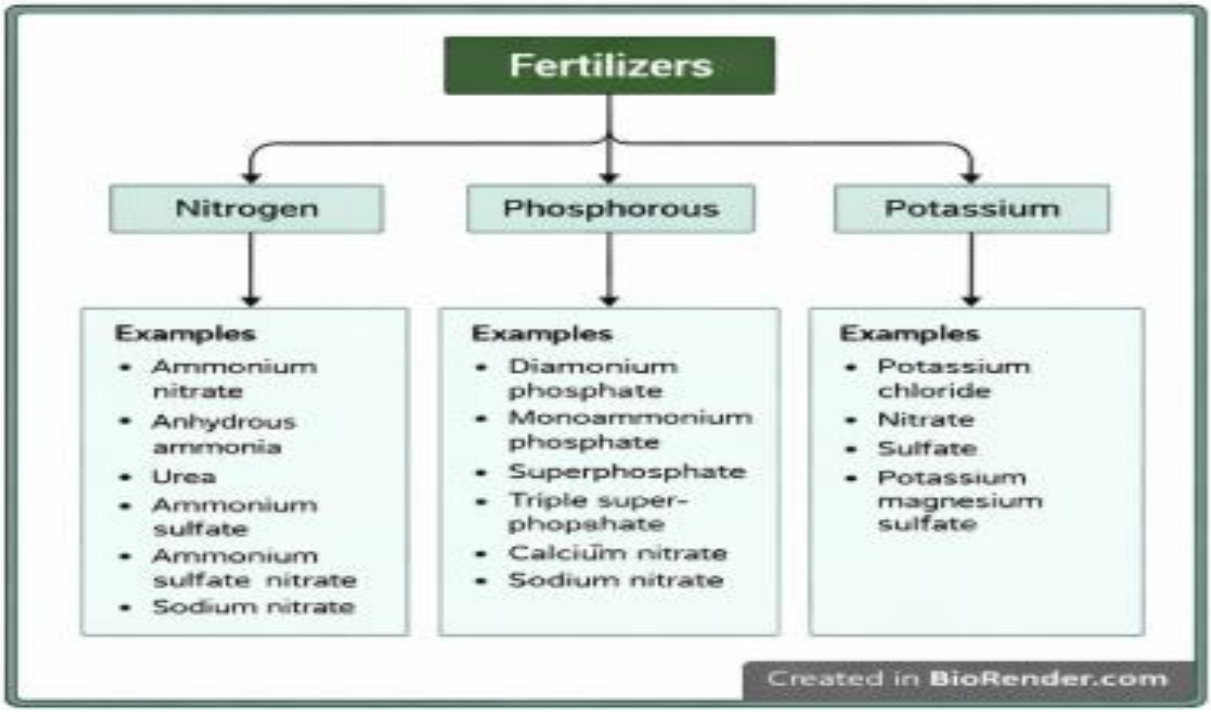
The study will provide valuable insights into the clinical implications of liver and renal dysfunctions among pesticide-exposed workers in the horticulture industry and may help guide practical solutions to address these health challenges. In addition, the identified associated factors offer important and immediate benefits for designing, targeting, and implementing effective agrochemical exposure education and prevention strategies. The findings will also be useful for planners, policymakers, and the broader community in developing interventions, preventive measures, and control programs for agrochemical exposure in Ethiopia, and particularly within the study area. Moreover, this study will serve as baseline information for future research.

2. Literature review

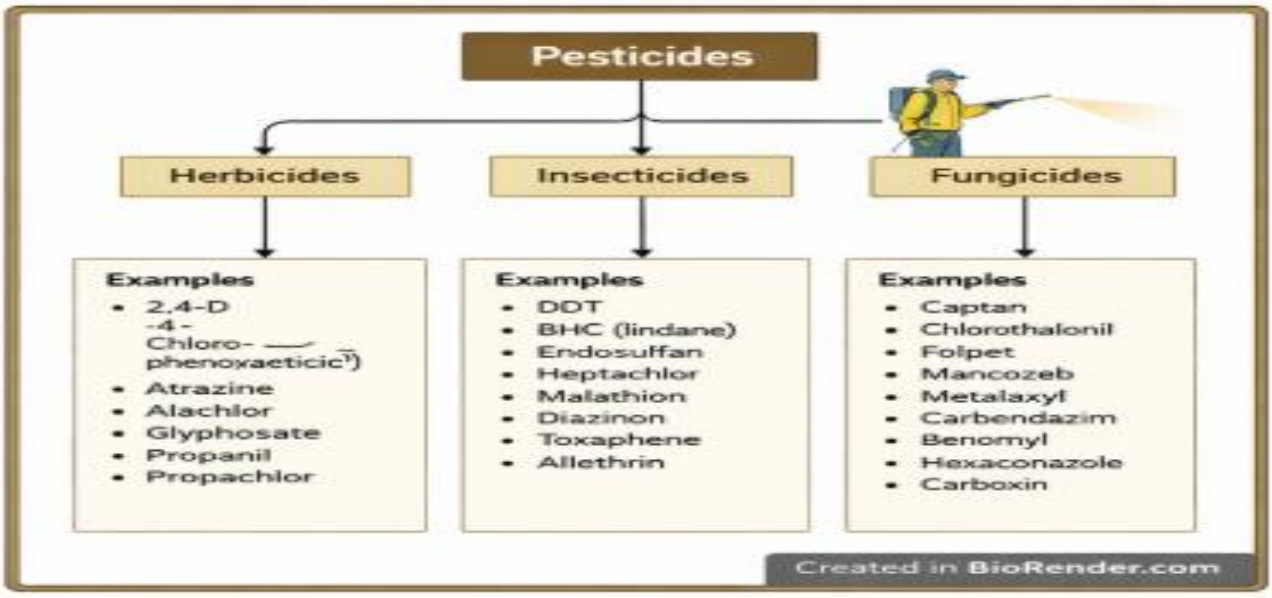
Pesticide contamination can adversely affect numerous enzymes, physiological processes, and mammalian organ systems, including the reproductive, nervous, immune, and endocrine systems, as well as blood coagulation, hematologic parameters, the cardiovascular system, sensory organs, skin, and the respiratory system. Additional consequences include risk for cancer and mutagenicity, fluid and electrolyte imbalance, and metabolic disruption. The liver is particularly vulnerable to the effects of pesticides, which can cause a wide range of alterations in several human organs. Particular pesticides can induce or inhibit particular enzymes, resulting in biochemical consequences. Workers exposed to pesticides have been reported to experience cholinesterase inhibition, liver and kidney damage, decreased hemoglobin levels, impaired oxidative stress responses with antioxidant imbalance, altered mixed-function oxidase activity, and disruptions in drug-metabolizing enzymes in the liver (48-50)

2.1. Agrochemicals, pesticides, and fertilizers

Agrochemicals include herbicides, fungicides, insecticides, nematicides, molluscides, rodenticides, and fertilizers (51)



A. Classification of Fertilizers



B. Classification of Pesticides

Figure 1: A: Classification of fertilizers; B: Classification of pesticides

Pesticides

Pesticides are environmental pollutants designed to eliminate unwanted living organisms, such as harmful animals or plants. Typically, a pest is defined as any organism that negatively interferes with human activities (52). According to Ethiopia's Ministry of Agriculture, the floriculture industry uses over 300 different chemicals, including insecticides, fungicides, nematicides, and growth regulators. About 120 of these chemicals are on the WHO's list of hazardous pesticides, and some are believed to be potentially carcinogenic. Despite the risks, such toxic substances are still commonly used in Ethiopia's flower farming sector (53). Based on their chemical structure, pesticides can be broadly classified into several groups, including Organochlorines, organophosphates, carbamates, pyrethroids, phenoxy-alkanoic acids, dipyridyls, phenylamines, triazines, benzoic acids, and phthalimides (54).

Table 1: Classification of pesticides based on their chemical nature

No	Chemical Group	Chemical Names
1	**Organochlorines**	Mirex, Endrin, Chlordecone, Telodrin, Isodrin, Perthane, Kepone, HCH (Hexachlorocyclohexane), Chlorthal-dimethyl
2	**Organophosphates**	Acephate, Chlorpyrifos, Ethion, Terbufos, Isazofos, Azinphos-methyl, Fonofos, Sulfotep, Etrimfos, Pirimiphos-methyl
3	**Carbamates**	Methomyl, Oxamyl, Carbofuran, Fenobucarb, Propoxur, Bendiocarb, Formetanate, Isoprocarb, Furathiocarb
4	**Pyrethroids**	Permethrin, Deltamethrin, Lambda-cyhalothrin, Esfenvalerate, Fenpropathrin, Bifenthrin, Tefluthrin, Tau-fluvalinate
5	**Phenyl amides**	Flufenacet, Meclobutanil, Fluopicolide, Carboxin, Benalaxyl, Metalaxyl, Boscalid, Pyrimethanil, Fenhexamid
6	**Phenoxy alkonates**	MCPA (2-methyl-4-chlorophenoxyacetic acid), MCPB, 2,4-DB, Dichlorprop-P, Fluoroxypyr, Picloram
7	**Trazines**	Prometryn, Terbuthylazine, Desmetryn, Cyanazine, Hexazinone
8	**Benzoic acid**	Benzoic acid methyl ester, Benzyl benzoate, Ioxynil, Bromoxynil

		octanoate
9	**Phthalimides**	Folpan, Captab, Phthalimidomethyl, Phthalimide-1-oxide
10	**Dipyrids**	Diquat dibromide, Paraquat dichloride, Bipyridyl
11	**Others**	Copper sulfate, Sodium chlorate, Boric acid, Iron phosphate, Silica gel, Pyrethrins, Neem oil, Spinosad, Abamectin, Beauveria bassiana (biopesticide)

Organophosphate

Every organophosphate insecticide has a core phosphorus (P) atom that is doubly linked to an oxygen or sulfur atom. Additionally, the central phosphorus (P) atom typically carries two methoxy or ethoxy groups singly attached to it, while a larger and more complex substituent (Z) is also bonded to the phosphorus usually through an oxygen or sulfur atom. (52). The toxicity of organophosphates is influenced by several factors, including the strength of the P–Z bond, the electrophilic character of the central phosphorus atom, the steric properties of the substituents (R_1 and R_2), and the presence of groups that increase the liability of the P–Z bond, which is cleaved during the inhibition process (55).

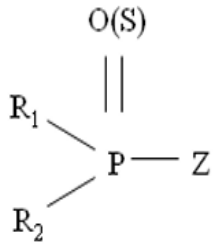


Figure 2: Structure of organophosphates

The most harmful organophosphorus insecticides have p-CH3–S–aromatic or p-NO2 aromatic groups because these substituents increase the P atom's electrophilicity through mesomeric and inductive effects. These groups undergo metabolic alteration (biotransformation) in mammals, which enhances the electrophilic nature of the phosphorus atom and subsequently increases the

toxicity of the pesticide. For example, a p-CH_3–S group can be oxidized to a p-CH_3–SO or p-CH_3–SO_2 group, both of which exert greater toxic effects. Because the electrophilic magnitude of the central phosphorus atom is reduced, the toxicity of organophosphate insecticides also diminishes as the chain length of the R1 and R2 groups increases. Because they reduce its enzymatic activity, organophosphate pesticides primarily target acetylcholinesterase, which results in the development of cholinergic toxicity. A hydroxymethyl group in the active site of the serine enzyme acetylcholinesterase reacts with organophosphate pesticides to create an inactive compound. (55-57).

Figure 3: Representation of biochemical interaction between organophosphates and acetylcholinesterase (Enzyme – OH) adopted from (58).

Acetylcholinesterase is weakly inhibited by organophosphate insecticides when a sulfur atom is attached to phosphorus (P = S) in the overall structure (59). When organophosphate insecticides (P = S) undergo phase I biotransformation processes, cytochromes P450 (CYP450) function as monooxigenases and desulfurize them to produce the equivalent oxon analogs (P = O). Because Oxon products can block acetylcholinesterase up to 1000 times more potently than their parent chemicals, the metabolic desulfurization process of organophosphorus insecticides is regarded as bioactivation (56, 60).

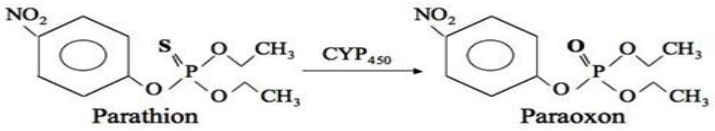


Figure 4: Biotransformation of parathion to paraoxon (bioactivation) (58)

Toxicity of pesticides

The Environmental Protection Agency (EPA) mandates toxicity tests in human health risk assessments (61). Pesticides go through several safety tests to assess health risks before approval. Acute toxicity tests evaluate short-term effects (within 1–2 days) from swallowing, skin contact, or inhalation, including irritation and nerve effects. Sub-chronic tests assess repeated exposure over 30 to 90 days, checking for organ or nervous system damage. Chronic toxicity tests span months to the animal's full lifespan, identifying long-term issues like cancer or organ failure. Developmental and reproductive tests examine effects on fertility and unborn offspring, typically conducted over gestation and reproductive cycles. Mutagenicity tests detect deoxyribonucleic acids (DNA) damage and the potential for genetic mutations, often conducted in short-term lab settings. Endocrine disruption tests study how pesticides may interfere with hormones over varied timeframes, depending on the system affected.

According to the toxicity of pesticides to human beings, the WHO has categorized them as follows: Class 1A is extremely dangerous, Class 1B is highly harmful, Class 2 is moderately hazardous, Class 3 is mildly hazardous, and Class U is unlikely to produce any hazards. The oral lethal dose 50 (LD50) in rats is the quantity required to kill 50% of the exposed rats in the event of an acute exposure, and animal experiments are the main source of the WHO classification. In toxicology, this classification scheme based on oral and dermal toxicity to rats is a common practice (62).

Table 2: Acute toxicity classification of pesticides

Class	**Hazard Description**	**Oral LD_{50} (mg/kg)**	**Dermal LD_{50} (mg/kg)**
Ia	Extremely hazardous	< 5	< 50
Ib	Highly hazardous	5 – 50	50 – 200
II	Moderately hazardous	50 – 2000	200 – 2000
III	Slightly hazardous	> 2000	> 2000
U	Unlikely to present a hazard	≥ 5000	≥ 5000

2.2. Knowledge, attitude, and practice of pesticide-exposed workers

According to a Thailand study on KAP of using PPE on 330 farmers who grow chili, 77.2% of study participants had low knowledge, only 22.8% had moderate knowledge, 54.5% had a poor attitude toward PPE (not concerned), 45.5% had a neutral attitude, and only 6% had good practice, while 94% had fair and poor practice (63). Another KAP study on pesticide exposure among farmers in Kota Bharu, Kelantan, Malaysia, in 2019 found that only 7.6% of the 144 study participants had high knowledge, 61.15% had moderate knowledge, and 31.3% had low (poor) knowledge. Only 4.5% had a concerned attitude, while about 56.35% and 38.9% had a not concerned attitude and a neutral attitude, respectively. Only 21.5% had good practice, while 63.2% had fair practice and 15.3% had poor practice (64).

According to a 2017 study on pesticide knowledge and safety practices among 250 farm workers in Kuwait, 64% of study participants did not complete training, and more than 70% did not read and heed label instructions. PPE is not used by 58% of research participants because it is not readily available when needed (35%), is too costly (65%), slows you down (29%), and is uncomfortable in the hot, humid atmosphere of the area (90%). The most often utilized PPE was goggles or glasses (48%), hats (42%), and protective gloves (61%) (65).

According to a 2022 study that assessed 299 farmers in western Bhutan's exposure to pesticides and KAP, only 17% had high knowledge, 23% had medium knowledge, and 59% had low (poor) knowledge. 63% of the study participants had a concerned (good) attitude, while 37% had no concern. Only 25% had good practice, with the remaining 40% and 35% having fair and poor practice, respectively (66).

A 2013 study on the KAP of 57 Indonesian farmers regarding the use of PPE to prevent pesticide exposure revealed that 40.4% of the participants had good knowledge and 60.6% had poor knowledge, that 35% of the participants had a good attitude and 65% had a poor attitude, and that 31.6% had good PPE practices while 68.4% had poor ones (67).

Numerous studies conducted in Ethiopia about KAP on pesticide exposure have been carried out. 3.09 % of sprayers met the body protection criteria, according to the first study, which evaluated

pesticide use and practice in Ethiopia's Bule Hora Districts with 413 study participants. Just 26% of respondents were able to understand and follow to instructions, despite 69% of them saying they could read labels on pesticide containers. As a result, 93.8% of farmers believed that pesticides were beneficial. 0.4% of farmers said pesticides were always harmful (68).

The second research conducted in 2019 on 409 farmers working in irrigation systems in Gondar town, Ethiopia, on the practices related to pesticide handling, storage, and related issues found that 36.2% of participants had high knowledge, and 63.8% had poor knowledge. 50.9% of people had a poor attitude, compared to 49.1% who had a good one. Of those that practiced, 36.2% were good and 63.8% were poor (69).

A study conducted in 2020 on pesticide-use KAP, and associated factors among 300 floriculture workers in Bahirdar, reported substantial gaps in safety awareness and practices. The study found that only 33.3% of participants had good knowledge, while 66.7% demonstrated poor knowledge, and 86.3% were unable to read or understand pesticide instructions. Regarding attitudes, 44.7% showed a positive attitude whereas 55.3% had a negative attitude toward safe pesticide use. In terms of practice, 61.3% exhibited good practices and 38.7% showed poor practices. Notably, 37.7% of workers never used personal protective equipment (PPE). Among PPE users, 59.3% wore gowns, 37.7% used gloves, 14.0% wore boots, and only 5.0% used facemasks during pesticide application. Furthermore, only 52.3% of workers reported receiving PPE from the floriculture farm, 64.7% ate and drank in the workplace, and 95.0% had not received any pesticide-related training before starting work (70).

An additional investigation conducted in 2017 on the low levels of occupational knowledge and practice among 471 flower farm workers in the southwest Shewa zone of Ethiopia demonstrated that 60.8% of them had poor knowledge, and 39.2% had high knowledge. 28% were participating in on-the-job training. As reported by 60.1% of respondents, they did not consistently use PPE (71).

2.3. Comparison of liver function tests among pesticide-exposed participants and controls

There was no statistically significant difference in ALT, AST, or ALP between pesticide-exposed orchid farmers and the control group, according to a 2015 cross-sectional study on the effects of pesticide exposure on immunological, hematological, and biochemical parameters in Thai orchid farmers (p values of 0.278, 0.175, and 0.056, respectively. In addition, there was a significant increase in total protein and albumin (p values <0.01) between pesticide-exposed farmers and controls (72). ALT (P-value <0.001), AST (P-value <0.001), and ALP (p-value - 0.036) were significantly higher than control in another recent study conducted in Thailand in 2023 on the Hematological and Biochemical Effects from Pesticide Use on Thai Vegetable Farmers (73).

A 2021 study on chronic organophosphate exposure in cohorts from Pakistan and Cameroon found significantly higher ALT and AST levels in exposed individuals than controls ($p < 0.01$). While there was no significant difference in ALP, TBL, and DBL observed between pesticide-exposed and controls in both countries (p-value>0.05) (74).

In 2020, a study on environmental pesticide residues and health biomarkers among farmers from Erbil cucumber crop greenhouses in Iraq found that greenhouse workers had significantly higher levels of the liver markers ALT, AST, ALP, DBL, and TBL (p-value $p<0.0001$) than control (75).

A 2018 study from Iran on hematological abnormalities, oxidative stress, and genotoxicity in greenhouse pesticide sprayers, found no significant differences in ALT or AST levels between sprayers and controls ($p = 0.211$ and 0.957, respectively) (76).

A 2019 Indian study on chronically pesticide-exposed agricultural workers found elevated ALT (16%), AST (12%), ALP (19%), and globulin (6%) ($p < 0.05$), along with reduced total protein and albumin (4.5%) ($p < 0.001$) compared with controls (77).

A 2020 Brazilian study found increased ALT ($p < 0.001$), AST ($p < 0.05$), and total protein ($p < 0.01$) in pesticide-exposed workers, with no significant differences in DBL or TBL compared with controls (78).

Elevation of ALT in pesticide-exposed farmers compared to controls, with p-value <0.01, was found in the study on evaluation of the effects of agro-pesticide use on liver and kidney function in farmers from Buea, Cameroon, in 2020. There were no notable changes in AST to the other parameters (79). A 2025 study from Cameroon on neurological and organ health risks in banana farm workers exposed to pesticide mixtures reported increased ALT, AST, and ALP levels ($p < 0.05$) (80).

A 2018 Benin study found significant increases in AST ($p < 0.001$) and ALT ($p < 0.05$) in pesticide-exposed vegetable farmers (81). A 2016 Tanzanian study on long-term pesticide exposure in female farm workers found increased ALT ($p < 0.01$) and decreased total protein ($p = 0.01$), with no significant difference in AST ($p = 0.08$) compared to controls (82). A 2019 Egyptian study on agricultural pesticide workers reported significant increases in ALT, AST, ALP, and total protein ($p < 0.001$) compared to controls (83).

2.4. Comparisons of renal function tests among pesticide-exposed participants and controls

A 2015 cross-sectional study on Thai orchid farmers found no significant differences in urea or creatinine levels between pesticide-exposed farmers and controls ($p = 0.729$ and 0.946, respectively) (72). A 2021 study in Nakhon Ratchasima, Thailand, on pesticide-induced changes in cholinesterase activity and chronic kidney disease reported that 27.6% of participants had abnormal creatinine and 24.1% had abnormal eGFR (84).

A study conducted in 2021 Chronic Exposure to Organophosphates Pesticides and Risk of Metabolic Disorder in Cohort from Pakistan and Cameroon indicated that significant increase in BUN (p. value <0.01) in Pakistan pesticide-exposed workers than controls, but not in Cameroon (p. value >0.05). Creatinine levels show a significant increase in pesticide-exposed individuals than controls in both countries (P-value <0.05) (74).

A 2020 study in Iraq on greenhouse cucumber farmers in Erbil reported a significant increase in urea ($p < 0.005$) among workers, with no significant change in creatinine compared to controls ($p = 0.5$) (75).

A 2018 study on greenhouse pesticide sprayers in Iran, investigating NQO1 gene polymorphism, found no significant differences in BUN or creatinine compared to controls ($p = 0.382$ and 0.161, respectively) (76).

A 2019 Indian study on chronic pesticide-exposed agricultural workers reported significant increases in BUN (15%, $p < 0.05$) and uric acid (15%, $p < 0.01$), with no significant changes in creatinine (4%) or BUN/creatinine ratio (7%) compared to controls (77). A study in the Gaza Strip on the effect of pesticides on farm workers' kidney function reported significant increases in urea and creatinine ($p < 0.05$) (85). A 2020 Brazilian study on occupational exposure to pesticides and metals reported significantly higher urea and creatinine levels in exposed workers compared to controls ($p < 0.01$) (78).

The findings of two investigations on Cameron, in 2016 and more recently in 2020, revealed the following. p values <0.001 and 0.001, respectively, for creatinine and uric acid increase in pesticide-exposed gardeners compared to controls in the first research, which was carried out in 2016 and examined changes in renal functions of market gardeners occupationally exposed to pesticides in West Cameroon (86). The second study in 2020 in Buea, Cameroon, on the effects of agro-pesticide use in farmers found no significant changes in renal function tests (79). A more recent study in 2025 in Moungo, Cameroon, found that pesticide-exposed banana farm workers had higher urea levels ($p < 0.05$) but no significant change in creatinine compared to controls (80).

A 2018 study in Benin on vegetable farmers found no significant changes in urea or creatinine levels ($p > 0.05$) due to pesticide exposure (81). A 2016 Tanzanian study on long-term pesticide exposure in female farm workers reported a significant increase in creatinine levels compared to controls ($p < 0.01$) (82). A 2019 Egyptian study on agricultural pesticide workers reported significant increases in urea and creatinine ($p < 0.001$) compared to controls (83).

2.5. Comparison of cholinesterase levels among pesticide-exposed and controls

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are encoded by separate genes on chromosomes 7 (7q22) and 3 (3q26), respectively, but they exhibit 65% amino acid sequence

similarity, analogous molecular forms, and a similar active site configuration (87). One of the quickest enzymes known, acetylcholinesterase (AChE), is primarily involved in the speedy breakdown of the neurotransmitter at cholinergic synapses. However, there is no physiological impairment associated with those BChE. In recent years, interest in its significance as a detoxifying enzyme has grown. Because BChE scavenges cholinesterase inhibitors, especially strong organophosphorus nerve poisons, and hydrolyzes ester-containing medications before they reach their synaptic targets, it is important for pharmacology and toxicology (88, 89).

AChE and BChE activity measurements are widely used as key biomarkers in occupational and emergency medicine to guide interventions in cases of clinical poisoning or accidental exposure to organophosphates (OP) and/or carbamates (CB) (90, 91).

Organophosphate (OP) exposure has been shown to affect cholinesterase activity primarily through BChE. This is partly because the degree of inhibition of AChE and BChE differs among various OP compounds, with some OPs exerting a stronger inhibitory effect on BChE. BChE inhibition is closely associated with the intensity and duration of exposure to a wide range of OP and CM pesticides. Additionally, factors such as the duration of exposure and the specific types of pesticides used contribute to significant variations in BChE activity and can be considered risk indicators of pesticide exposure (92).

However, BChE inhibition does not accurately reflect the neurotoxic effects of organophosphates on the nervous system (93). In contrast, AChE is a more reliable indicator than BChE for assessing chronic OP exposure, as its slower recovery rate leads to cumulative inhibition over time (94).

A 2015 study on Thai orchid farmers found that pesticide-exposed individuals had significantly lower BChE levels compared to controls ($p < 0.001$) (72). A 2021 study in Nakhon Ratchasima, Thailand, reported that 46.5% of farmers exposed to pesticides had abnormal BChE levels (84). A 2021 study on chronic organophosphate exposure in cohorts from Pakistan and Cameroon reported significant decreases in AChE ($p < 0.01$) and BChE ($p < 0.05$) levels in pesticide-exposed individuals compared to controls in both countries (74). A 2020 study in Iraq on greenhouse cucumber farmers in Erbil found a significant decrease in BChE levels in pesticide-exposed workers compared to controls ($p < 0.0001$) (75).

2018 Iranian study on greenhouse pesticide sprayers, investigating NQO1 gene polymorphism, reported a significant 28% reduction in BChE levels compared to controls ($p < 0.001$) (76). A 2025 study in Moungo Division, Cameroon, reported a significant decrease in BChE levels in pesticide-exposed banana farm workers compared to controls ($p < 0.05$) (80).

2.6. Associated factors of liver function tests, renal function tests, and butyrylcholinesterase

A 2021 study in Nakhon Ratchasima, Thailand, found that pesticide-exposed farmers who did not use PPE had higher odds of abnormal eGFR (84). A study in the Gaza Strip on pesticide exposure found that longer work duration was associated with higher urea and creatinine levels in farm workers (85).

A study in 2009 in Brazil on Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity indicated that pesticide-exposed participants did not use PPE, a significant decrease in BChE than participants who used PPE (p. value <0.050) (95).

A study in 2014 in Tunisia on oxidative stress, hematological, and biochemical changes in farmers exposed to pesticides showed that a significant negative correlation between BChE and years of pesticide exposure (r = -0.406, p-value - 0.004) (96).

A study in 2014 in Egypt on the Influence of exposure to pesticides on liver enzymes and cholinesterase levels in male agriculture workers showed that significant negative correlation between age and BChE (r = -0.220, P. value – 0.028), there was significance positive correlation TBL and age (r = 0.216, P. value -0.031) (97).

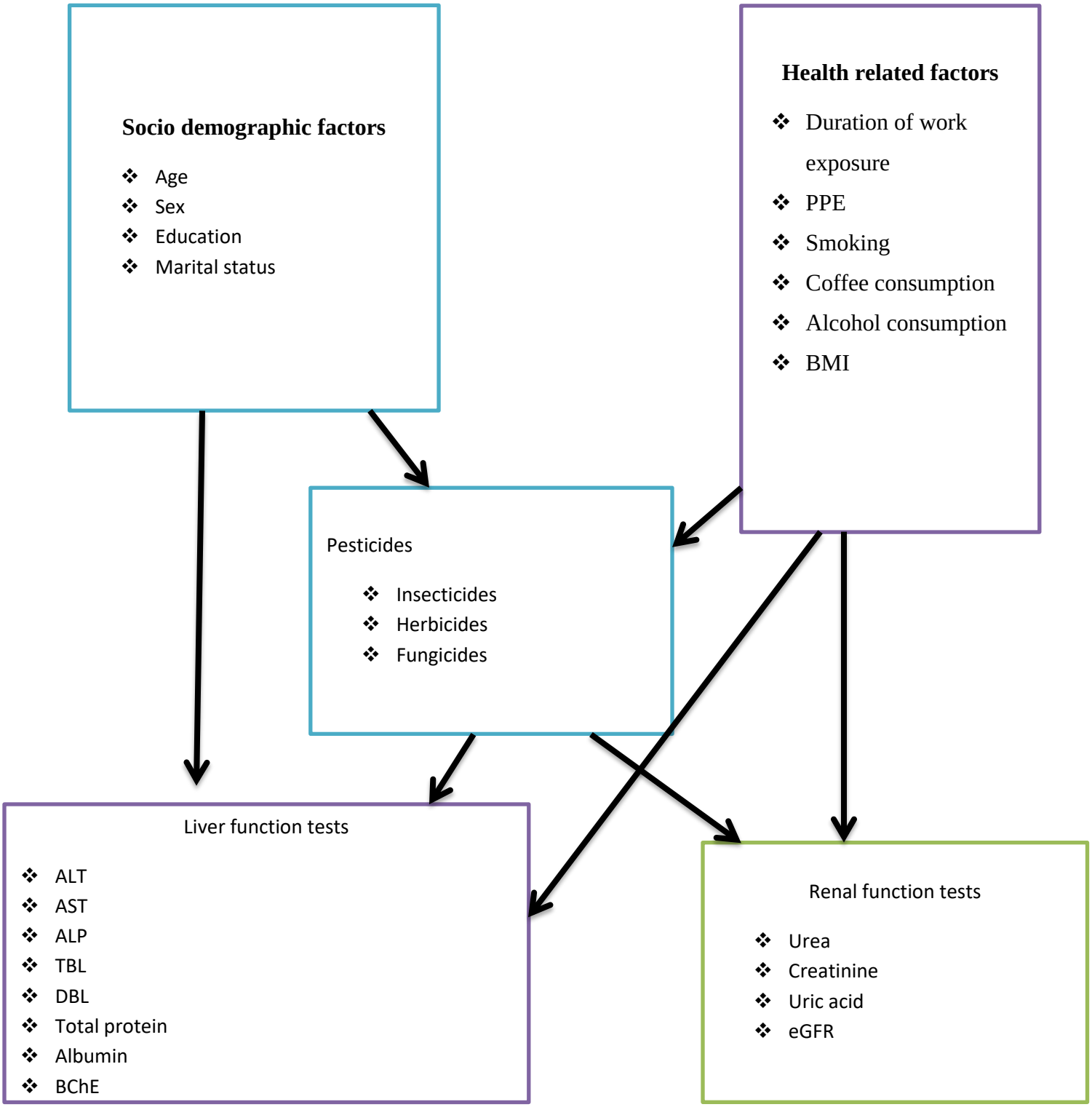


Figure 5: Conceptual framework of the study, summarized from the literature review

3. Objectives

3.1. General objective

- To assess liver function tests, renal function tests, serum cholinesterase level, knowledge, attitude and practice among horticulture workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

3.2. Specific objectives

- To compare liver and renal parameters of horticulture workers with controls at Tana flora floriculture, Bahirdar, Ethiopia, 2025.
- To compare Serum cholinesterase level of horticulture workers with controls at Tana flora floriculture, Bahirdar, Ethiopia, 2025.
- To assess factors associated with liver and renal dysfunction among horticultural workers at Tana flora floriculture, Bahirdar, Ethiopia, 2025.
- To assess knowledge, attitude, and practice of workers toward agrochemical exposure at Tana flora floriculture, Bahirdar, Ethiopia, 2025

4. Hypothesis

H0: - There is no difference between the means of liver function tests, renal function tests, and serum cholinesterase between horticultural workers and controls in the total population at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

-There is no significant association between associated factors among horticultural workers at Tana flora Floriculture industry, Bahirdar, Ethiopia, 2025.

-There is no Knowledge, Attitude, and Practice gap among horticultural workers at Tana flora Floriculture industry, Bahirdar, Ethiopia, 2025.

5. Materials and Methods

5.1. Study area

Bahirdar is located 563 kilometers from Addis Ababa, Ethiopia's capital. Both rural and urban residents reside in this city. Four private hospitals, 56 private specialty clinics, 13 private medium clinics, 11 healthcare facilities, 15 health posts, and three public hospitals can all be found in the city (98). According to the 2007 Central Statistical Agency of Ethiopia (CSA) Census, 221,991 people were living in the Bahir Dar Special Zone, with 108,456 men and 113,535 women. The remaining individuals were dispersed among the rural kebeles surrounding Bahir Dar, with 180,174, or 81.16%, residing in urban areas. The three largest ethnic groups identified in the Bahir Dar Special Zone were the Amhara (96.23%), Tigrayans (1.11%), and Oromo (1.10%); the remaining ethnic groups made up 1.56% of the total population.

Geographically, Tana Flora is located at latitude of 11°35'30" North and longitude 37°23'30" East. It is located in the Amhara Region of Ethiopia's Wonjeta Kebele, Bahir Dar Zuria region. On June 22, 2009, Tis Isat Water Works and Gafat Endowment founded it. The distances are 582 kilometers to Addis Ababa and 18 kilometers to Bahir Dar. Rainfall there averages between 800 and 1200 mm annually. The area has 124 hectares, 40 of which are allocated for growing fresh cut roses in greenhouses for the world's flower industry. Because of floriculture, 950 people now have jobs, 70% of them are women. It contributes to the country's economic growth by selling cut flowers, bringing in an average of $10 million USD annually (99, 100).

The controls were selected from the small town of Zege. Zege is a rural satellite town of the Bahr-Dar city government, located 32 kilometers from the Amara National Regional capital, Bahir Dar (101). According to the city government of Bahir Dar's 2019 population projection, Zege now has 10,083 residents (4041 men and 6042 women). Zege contains three Keble’s and 2142 households overall, 709 of which garden at home and 1433 of whom do not. Zege has three health posts and one health center, and each of these facilities provides medical care to the local population as well as those in the surrounding area. The Tana Flora floriculture company is 15 kilometers away from Zege rural town (102).

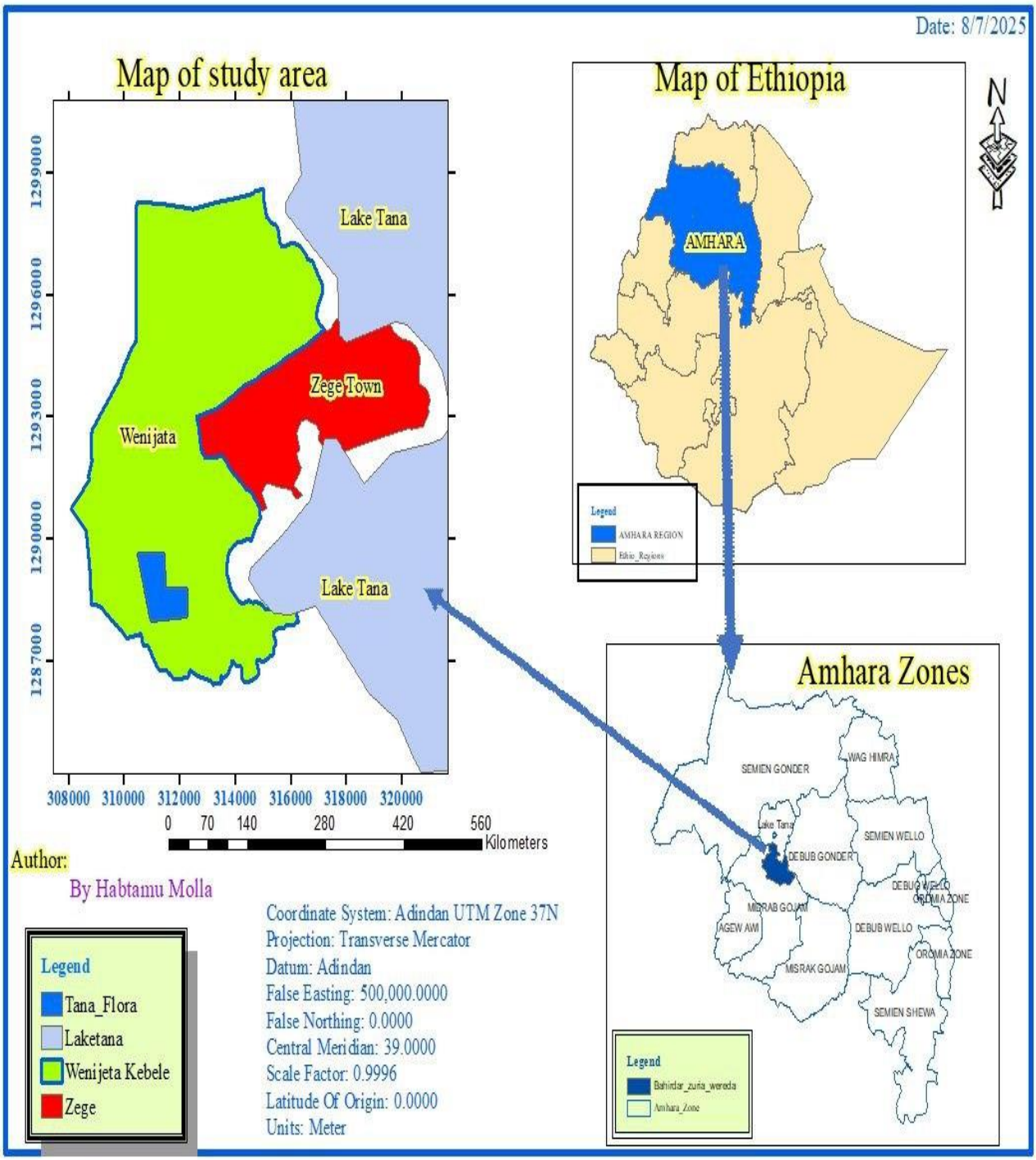


Figure 6: Map of the study area (source: own Arc Geographical information system (GIS design)

5.2. Study design and periods

A comparative cross-sectional study was conducted from February 8, 2025, to May 28, 2025.

5.3. Population

5.3.1. Source population

- **Exposed group**: All employees of Tana Flora Floriculture Industry
- **Control**: All resident of zege town

5.3.2. Study Population

- **Exposed group**: All active employees of Tana Flora Floriculture at the time of the study
- **Control:** Age and sex matched residents of zege town

5.4. Inclusion and Exclusion Criteria

5.4.1. Inclusion Criteria

Exposed group: -

- Workers who have been working a minimum of six months in the horticulture site and are permanently employed
- Workers who voluntarily participated in the study and signed for consent form

Control group: –

- Apparently healthy residents of the Zege satellite town that were volunteers and matched with exposed participants in age, sex, and not exposed to pesticides.

5.4.2. Exclusion Criteria

- Workers who have a history of renal and liver health problems for both the control and the exposed group
- Pregnant women's
- Administrative staff and managers

5.5. Study Variables

5.5.1. Dependent Variables

- Liver and renal function tests
- Serum cholinesterase

5.5.2. Independent Variables

- Socio-demographic characteristics (age, sex, education, marital status)
- Duration of work exposure
- Personal protective measures
- Types of pesticides
- Alcohol consumption
- Coffee consumption
- BMI
- Periodical check up
- On job training
- Knowledge, attitude and practice

5.6. Sample size Determination and sampling procedure

5.6.1. Sample size calculation

According to the authors' understanding, there has been no prior publication addressing on the assessment of liver and renal function tests among horticultural workers in Ethiopia. The sample size for the study was calculated using G*Power version 3.1.9.7 software, applying a two-tailed t-test with the following parameters: α = 0.05, power (1-β) = 0.8 (80%), a 1:1 ratio of floriculture workers to controls, and a medium effect size (d) = 0.55. The calculation was performed using the formula:

$$\mathbf{N = \frac{2(Z\beta + Z\alpha/2)^2}{d^2}}$$

Where: - N- Number of participants in each group, $\boldsymbol{Z\beta}$- (B= 20%)-0.84, $\boldsymbol{Z\alpha/2}$- ($\boldsymbol{\alpha}$=0.05)-1.96, d-effect size-0.55·

$$N = \frac{2(0.84+1.96)^2}{(0.55)^2} = 15.68/\ 0.3025 = 52$$

A totals of 104 participants (52 in floriculture worker and 52 pesticide non exposed controls) were enrolled in the study.

5.6.2. Sampling procedure

A floriculture farm includes a majority of 4 areas: green houses, pack houses, irrigation, and pesticide spraying. Tasks in the greenhouse include building flower beds, applying fertilizers and pesticides, planting, weeding, pruning, harvesting flowers, and transporting organic waste. Employees in this environment are at higher risk of pesticide exposure due to prolonged work in an enclosed space with high chemical concentrations. In the spraying department, pesticides are typically mixed and manually sprayed using spray lances. Walking into the spray mist while manually spraying with spray lances increases exposure to pesticides through the skin and respiratory systems (103).

The pack house is where post-harvest operations take place, and gathered flowers are arranged for export. It's likely that workers in the pack house were exposed to high pesticide doses while carrying out their tasks in an enclosed space to preserve flower quality. The irrigation section was in responsible for monitoring water lines and mixing fertilizers and other substances required for flower growth. Workers in this department combine different fertilizer ingredients (104).

In order to choose the cases for the study, a proportionate simple random and stratified selection method was used. There were 591 active workers in the floriculture sector (218 men and 373 women). 37 employees (25 men and 12 women) were managers and administrative staff and were not included in the research. 64 workers (24 men and 40 women) were temporary employees who worked for less than six months and were not included in the study. The study includes the remaining 490 employees. Out of 490 employees, 320 worked in the greenhouse (cutting flowers), 126 in packing, 33 in spraying, and 11 in irrigation. 52 study participants were recruited for the study. One Greenhouse female participant was excluded from the study because to a known liver disease. As seen in the image below, the final sample size was 51 (15 males and 36 females). The controls were chosen using purposeful sampling from the zege town households from 3 kebeles. 18 households selected from Kebele, 17 households from Kebele 2 and 17 households from Kebele 3. From each house hold one participant purposively selected which was age and sex matched with our case. 52 controls (34 females and 18 male) were

recruited for the study. One female participant's know liver disease excluded from the study. The control group's final sample size was 51 (33 females and 18 males).

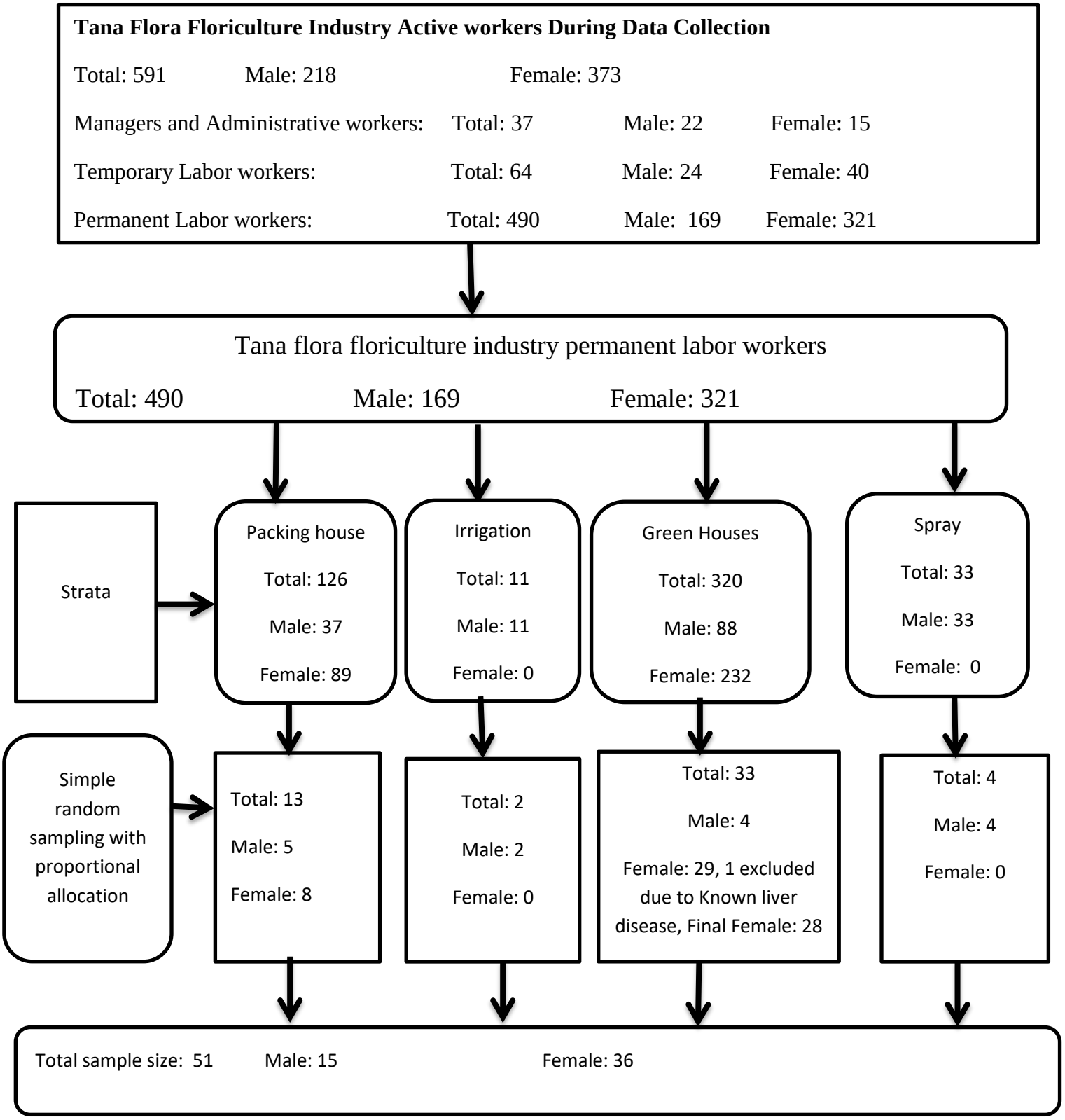


Figure 7: Sampling procedure of assessment of liver and renal function tests among horticultural workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

5.7. Measurement and Data Collection

5.7.1. Sociodemographic and associated factor Data collection procedure

Socio-demographic data and associated factors were collected through face-to-face interviews using a questionnaire. The questionnaire, adapted and modified from various English-language sources, was translated into local languages by the interviewers to ensure comprehension and minimize data collection bias. Data were then obtained using both the translated questionnaire in interviews and self-administered forms. The questionnaire included both closed- and open-ended questions, covering socio-demographic information, employment duration, knowledge, attitude, and practices regarding pesticide exposure, protective measures, health problems, pesticide-related symptoms, and lifestyle factors such as smoking, coffee, and alcohol consumption. Participants' weight was measured using digital scales and height in meters, and BMI was calculated from weight (kg) divided by height squared (m^2).

5.7.2. Assessments of Knowledge, Attitude and practice of floriculture workers towards pesticide exposure

Knowledge, attitude, and practice (KAP) scores were calculated by summing item responses, with scores categorized as good or poor. The questionnaire included 4 knowledge items, 5 attitude items, and 8 practice items.

To assess knowledge, four items were included: (i) knowledge of pesticide names, (ii) ability to read and understand pesticide labels, (iii) awareness of pesticide-related health problems, and (iv) understanding of routes of exposure. Responses were coded as 1 for correct (Yes) and 0 for incorrect (No). Knowledge scores were then categorized as "Good" or "Poor."

Workers' attitudes toward pesticide use were assessed using a 5-item, 4-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = agree, 4 = strongly agree). The items included: (i) all pesticides have adverse effects on human health, (ii) pesticide use should be discouraged, (iii) the

body has resistance to pesticides, (iv) PPE use prevents pesticide exposure, and (v) proper pesticide handling reduces health risks. Attitude scores were finally categorized as “Positive” or “Negative.”

To assess the practice on pesticide use, detailed lists of knowledge questions were (8 items such as: (i) usage of PPE, (ii) taking a bath after pesticides are applied, (iii) washing hands immediately after work, (iv) changing clothes before going home, (v) consumption of coffee, (vi) consumption of alcohol, (vii) action taken after pesticide exposure, and (viii) habit of eating, drinking, chewing in work place. Responses were coded such that correct answers (Yes) scored 1 and incorrect (No) except consumption of coffee, consumption of alcohols, and habit of eating, drinking and chewing in work area were reversibly coded (Yes) scored 0 and (No) scored 1. Action taken after pesticide exposure coded as (Traditional medicine) scored 1, Stop work and rest scored 2 and go to health facility scored 3. Finally, practice related questions were categorized in to 2 categories as “Good and “Poor”

Table 3: Classification of knowledge, attitude, and practice.

Score values (Total)	Knowledge	Attitude	Practice
>50%	Good	Positive	Good
≤ 50 %	Poor	Negative	Bad

The internal consistency of the 4-item knowledge question was good (Cronbach’s $\alpha = 0.890$; standardized $\alpha = 0.892$). The internal consistency of the 5-item attitude Likert questions was excellent (Cronbach’s $\alpha = 0.972$; standardized $\alpha=0.972$). The internal consistency of 8 items was acceptable (Cronbach’s $\alpha= 0.72$; standardized $\alpha= 0.73$). Table 3 shows the cut off values classification of knowledge, attitude and practices.

5.7.3. Specimen Collection and Processing

After obtaining written informed consent and completing the questionnaire, participants were registered and assigned a unique serial number. Blood samples were collected voluntarily, with 5 mL of venous blood drawn from the medial cubital vein using an SST tube, following disinfection with 70% alcohol by the principal investigator and trained laboratory personnel. The

samples were left at room temperature for 10–20 minutes to allow coagulation. The blood samples were transported to the International Clinical Laboratories (ICL) in an ice-cold box within 8 hours and centrifuged at 3,000 rpm for 5 minutes to separate the serum from the cells. The serum was then transferred to Nunc tubes and stored in a deep freezer at –70 °C. After all specimens were collected and preserved in a deep freeze, all the samples were transported and analyzed at the Ethiopian Public Health Institute (EPHI). The specimen will be analyzed by the principal investigator and EPHI National Reference Clinical Chemistry laboratory workers. Liver function (ALT, AST, ALP, TBL, Total protein, Albumin, and DBL), RFT (Urea, Creatinine, Uric acid), and BChE were done on the EPHI National Clinical Chemistry Reference Laboratory by Cobas 6000 clinical chemistry analyzer.

5.7.3. Laboratory analysis

5.7.3.1. Liver functional tests

LFTs, including ALT, AST, ALP, total protein, TBL, DBL, and albumin, were analyzed. These biochemical markers were measured spectrophotometrically using analytical-grade laboratory reagent kits.. The laboratory reagent kits from Roche were used to assess the concentration of ALT (IFCC without pyridoxal phosphate method (105)), AST (IFCC without pyridoxal phosphate method (105)), ALP(IFCC P-Nitro Phenyl Phosphate substrate method (106)) , Total protein (Biuret reaction method (107)) TBL (Diazo reaction (108)), DBL (Diazo reaction (109)), and Albumin (BCG method (110)) in the serum. All LFTs were measured using the Cobas 6000 automated clinical chemistry analyzer with the corresponding reagent kits, following the manufacturer's protocol (Full principles of the laboratory tests described in annex V).

5.7.3.2. Renal Function tests

RFTs, including urea, creatinine, and uric acid, were analyzed. These biochemical markers were measured spectrophotometrically using analytical-grade laboratory reagent kits.. The laboratory reagent kits from Roche were used to assess the concentration of urea (Urease and glutamate dehydrogenase kinetic test(111)), creatinine (Enzymatic method (112)), and uric acid (Uricase method)in the serum. All RFTs were measured using the Cobas 6000 automated clinical

chemistry analyzer with the manufacturer's reagent kits, following the provided protocol (Full laboratory principles of tests described in annex V).

5.7.3.3. Serum Cholinesterase

BChE (EC 3.1.1.8) was measured by Schmidt et al method (113) in a Cobas 6000 automated clinical analyzer by spectrophotometric determination of its absorbance using analytical grade laboratory reagent kits. The laboratory reagent kit from Roche was used to assess the activity of serum cholinesterase. It was analyzed by its own reagent kit. The biochemical analysis for this study was performed according to the manufacturer. Measuring range of the test is 100-14000 U/L. Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision and CV was 0.5%. (The full principles of tests described in annex V).

5.7.4. Calculations

5.7.4.1. Creatinine-based eGFR

The 2021 CKD-EPI creatinine equation is endorsed by the National Kidney Foundation and the American Society of Nephrology as the preferred method for estimating eGFR in U.S. adults, replacing previous equations (114).

$$\mathbf{eGFR = 142 \times \min(SCr/k, 1)^{a} \times \max(SCr/k, 1)^{-1.200} \times 0.9938^{Age} \times 1.012\ [if\ female]}$$

Where: SCr is serum creatinine in mg/dL, k- 0.7 (females) or 0.9 (males), a -0.241 (female) or -0.302 (male), Scr - serum creatinine in mg/dL, min indicates the minimum of SCr /k or 1, and max indicates the maximum of SCr /k or 1(115).

Reference range of EGFR

The six CKD categories, labeled stages 1 through 5 (with stage 3 further divided into 3a and 3b), are described as follows (116):

- G1: GFR ≥ 90 mL/min/1.73 m² with evidence of kidney damage (e.g., hematuria or proteinuria)

- G2: GFR 60–89 mL/min/1.73 m² – Mildly decreased (may be normal)
- G3a: GFR 45–59 mL/min/1.73 m² – Mild to moderate decrease
- G3b: GFR 30–44 mL/min/1.73 m² – Moderate to severe decrease
- G4: GFR 15–29 mL/min/1.73 m² – Severely decreased
- G5: GFR < 15 mL/min/1.73 m² – Kidney failure (End-Stage Renal Disease)

5.7.5. Interpretation of results

Results were interpreted using the normal reference ranges provided by the test kits. Each analyte was assessed according to the established adult reference values.

Table 4: Reference ranges of liver function, renal function, and BChE.

S. No	Tests	Sex	Kit Reference ranges	References
1	ALT	Adult Male	<41 U/L	(117)
		Adult Female	<33 U/L	
2	AST	Adult Male	<40 U/L	(117)
		Adult Female	<31 U/L	
3	ALP	Adult Male	40-129 U/L	(118)
		Adult Female	30-104 U/L	
4	Total protein	Adult	6.6 -8.7 g/dl(66-87 g/l)	(119)
5	Albumin	Adult	3.5-5.2 g/dl (35-52 g/l)	(120)
6	Globulin	Adult	2- 3.5 g/dl (20-35 g/l)	(121)
6	TBL	Adult Male	<1.4 mg/dl	(122)
		Adult Female	< 0.9 mg/dl	
7	DBL	Adult	<0.30 mg/dl	(123)
8	Urea	Adult	16.6 mg/dl-48.5 mg/dl	(122)
9	BUN	Adult	6-20 mg/dl	(124)
10	Creatinine	Adult Male	0.70-1.2 mg/dl	(125)

		Adult Female	0.50-0.90	
11	Uric acid	Adult Male	3.4-7.0 mg/dl	(126)
		Adult Female	2.4-5.7 mg/dl	
12	BChE	Adult Male	5320-12920 U/L	(127)
		Adult Female	4260-11250 U/L	
13	BUN to creatinine ratio	Adult	10:1 to 20:1	(128)
14	A/G ratio	Adult	1.0 - 2.0	(129)

5.8. Data Quality Assurance

5.8.1. Pre-analytical

Data quality was ensured by using a validated and pretested questionnaire. A pretest was conducted on 5% of the study population at Ethio Agriceft Floriculture Industry, not included in the main study, and necessary adjustments were made based on the findings. Data collectors received one day of intensive training on the study instrument and procedures, covering the study's relevance, objectives, confidentiality, informed consent, and interview techniques. They worked under close supervision to ensure proper data collection, and the principal investigator reviewed completed questionnaires daily for completeness. Morning sessions were held to address any issues promptly and implement corrective measures. Additionally, the data were carefully entered and cleaned prior to analysis.

All procedures, including blood collection, transportation, and storage, followed good laboratory practices (GLP) and standard operating procedures (SOPs) to ensure data quality. Participants were prepared and samples labeled from PE001–PE51 and NPE001–NPE051 on both the questionnaire and SST tubes. Venous blood was drawn from the antecubital fossa after disinfection with 70% alcohol and placed in plain tubes with a separator gel. Specimens were then transported to the laboratory, centrifuged to separate serum, and analyzed.

5.8.2. Analytical

The test was analyzed EPHI National Clinical Reference Laboratory. The equipment was calibrated monthly using the type-Auto calibrator, and two levels of internal quality control (IQC) samples normal and pathological were run alongside each serum sample. The EPHI National Clinical Chemistry Reference Laboratory received accreditation from ENAO on February 17, 2022. The accreditation is awarded to 16 tests, which are ALT, AST, ALP, LDH, Albumin, Alpha amylase, Cholesterol, TG, Urea, Creatinine, Total protein, Uric acid, Glucose, TBL, DBL, and Phosphate in accordance with the requirements of ISO 15189: 2013. Control sample results were interpreted using the Westgard multi-rule algorithm, and each analyte was analyzed by the principal investigator and senior laboratory technologists after carefully reviewing the corresponding protocol.

5.8.3. Post-analytical

After analysis, the printed results were reviewed for post-analytical factors, including reporting units and correct serial numbers assigned by the investigator. The results were then approved by the responsible laboratory technologist and promptly delivered to the principal investigator.

5.9. Data Analysis and Interpretation

Data were manually checked for completeness, edited, and entered into Epidata 4.7, then analyzed using SPSS version 27 for Windows® (SPSS Inc., Chicago, IL, USA). After cleaning and organizing, frequencies and percentages were calculated for variables related to the study objectives. Categorical variables were analyzed using the chi-square test, and independent t-tests assessed mean differences between exposed and control groups. The Kruskual-Wallis test was used to evaluate the effect of exposure duration on liver and renal function tests and cholinesterase levels. Normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, with $p > 0.05$ indicating normal distribution. Spearman correlation and stepwise multiple linear regression were applied to identify risk factors associated with LFTs, RFTs, and BChE. Statistical significance was set at $p < 0.05$ with a 95% confidence interval. Results were presented in tables and narrative form.

5.10. Ethical considerations

The study was ethically reviewed and approved by the Department of Medical Laboratory Science Research and Ethics Review Committee, College of Health Science, Addis Ababa University. Permission to assess liver and renal function tests among pesticide-exposed horticultural workers was obtained from the Tana Flora Floriculture administration. Participants were informed about the study's purpose, assured of confidentiality, and informed that participation was voluntary. Data collection commenced after receiving administrative approval, and signed consent was obtained from all participants. To maintain confidentiality, no personal identifiers were used, and the data were securely stored and accessed only by the principal investigator.

5.11. Operational Definitions

- **Hepatotoxicity**: is toxicity to the liver, bile duct, and gallbladder
- **Liver function Tests**: are a group of blood tests used to evaluate and manage patients with hepatic dysfunction. Commonly assessed parameters include ALT, AST, ALP, total bilirubin (TBL), direct bilirubin (DBL), albumin, total protein, globulin, and the albumin-to-globulin (A/G) ratio.
- **Nephrotoxicity:** is toxicity to the urinary tract system.
- **Renal Function Tests**: are a group of blood tests used to evaluate and manage patients with kidney dysfunction. Common parameters include urea, creatinine, eGFR, uric acid, and the BUN-to-creatinine ratio.
- **Horticultural worker**: Workers who have been engaged for over 6 months in the floriculture industry.
- **BMI**: was calculated from weight and height and categorized as <18.5 kg/m² (Underweight), 18.5–24.9 kg/m² (Normal), 25.0–29.9 kg/m² (Overweight), and ≥30 kg/m² (Obese)) (130).
- The Tropical Pesticide Research Institute (TPRI) Act No. 18 of 1979 describes pesticides as "any matter of any description (including acaricides, arboricides, herbicides, insecticides, fungicides, molluscides, nematicides, hormonal sprays, and defoliants) used or intended to be used, either alone or together with other material substances, for the control of weeds, pests,

and diseases in plants, or for the control of external parasites of man or domestic animals, as well as for the protection of any food intended for human consumption "(6).

- **BChE level:** *Low BChE level* – BChE less than 5320 U/L and 4260 U/L for adult males and females, respectively; *Normal BChE level* – BChE between 5320 U/L and 12920U/L and 4260 U/L to 11250 U/L for adult males and females, respectively; *High BChE level* – BChE greater than 12920 U/L and 11250U/L for adult males and females, respectively (127).
- **CKD levels by eGFR CKD-EPI-2021:** *Stage 1*- eGFR greater than 90 mL/min/1.73m^2; *Stage 2* – eGFR between 60 mL/min/1.73m^2 and 89mL/min/1.73m^2*; Stage 3a* – eGFR between 45 mL/min/1.73m^2 and 59 mL/min/1.73m^2; *Stage 3b* – eGFR between 30 mL/min/1.73m^2 and 44 mL/min/1.73m^2; Stage 4 – eGFR between 15 mL/min/1.73m^2 29 mL/min/1.73m$^{2;}$ Stage 5 – eGFR less 15 mL/min/1.73m^2 (131).
- **AST level:** *Normal AST level* - AST less than 40 U/L and 32 U/L for adult male and female, respectively; *High AST level*- AST greater than or equal to 40.00 U/L and 32.00 U/L for adult male and female, respectively (117).
- **ALP level:** *Low ALP level* -ALP less than 40 U/L and 30 U/L for adult male and female respectively; *Normal ALP level*- ALP between 40 U/L to 129 U/L and 30 U/L to 104 U/L for adult male and female respectively; *High ALP level*- ALP greater than 129 U/L and 104 U/L for adult male and female respectively (118).
- **TBL level:** *Normal TBL level* -TBL less than 1.4mg/dl and 0.9 mg/dl for adult male and female, respectively; *High TBL*- TBL greater than or equal to 1.4 mg/dl and 0.9 mg/dl for adult male and female, respectively (122).
- **DBL level:** *Normal DBL level* –DBL less than 0.3 mg/dl for adult males and females; *High DBL* – DBL greater than or equal to 0.3 mg/dl for adult males and females (123).
- **TP level –** *Low TP level* – TP less than 6.6 mg/dl for adult male and female; *Normal TP level* – TP between 6.6 mg/dl to 8.7 mg/dl for adult male and female; *High TP level* – TP greater than 8.7 mg/dl for adult male and female (119).
- **Albumin level:** *Low albumin level* - Albumin less than 3.50 mg/dl for males and females; *Normal Albumin level* - Albumin between 3.50 mg/dl to 5.20 mg/dl for adult males and females; *High Albumin level*- Albumin greater than 5.20 mg/dl for adult males and females (120).

- **Globulin level:** *Low globulin level*- Globulin less than 2.00 mg/dl for adult male and female; *Normal globulin level*- Globulin between 2.00 mg/dl to 3.50 mg/dl for adult male and female; *High globulin level*- Globulin greater than 3.50 mg/dl for adult male and female (120).
- **Urea level:** *Normal urea level*- Urea less than 48.5 mg/dl for adult males and females; *High urea level* – Urea greater than or equal to 48.500 mg/dl for males and females (122).
- **Creatinine level:** *Normal creatinine level* - Creatinine less than 1.20 mg/dl and 0.90 mg/dl for adult male and female, respectively; *High creatinine level*- Creatinine greater than or equal to 1.20 mg/dl and 0.90 mg/dl for male and female, respectively (125).
- **Uric acid level:** *Normal uric acid level* – Uric acid less than 7.00 mg/dl and 5.70 mg/dl for adult male and female, respectively; *High uric acid level*- Uric acid greater than or equal to 7.00 mg/dl and 5.70 mg/dl for male and female, respectively(126).
- **BUN level:** *Normal BUN level* – BUN less than 20 mg/dl for adult males and females; *High BUN level* – BUN greater than or equal to 20 mg/dl for adult males and females (124).
- **BUN to creatinine ratio level:** *Low BUN to creatinine level*- BUN to creatinine less than 10:1 for adult male and females; *Norma BUN to creatinine level*- BUN to creatinine ratio 10:1 to 20:1 for adult males and females; *High Bun to creatinine ratio level* – BUN to creatinine ratio greater than 20:1 for adult male and female (128).
- **A/G ratio level:** *Low albumin to globulin ratio level* – Albumin to globulin ratio less than 1.0 for adult male and females; *Normal albumin to globulin ratio level*- Albumin to globulin ratio between 1.0 to 2.0 for adult male and female; *High albumin to globulin ratio level* –Albumin to globulin ratio greater than 2.0 for adult male and female(129).

5.12. Result dissemination

The findings of this study will be presented to the Department of Medical Laboratory Science, Addis Ababa University, and may serve as reference material for researchers, experts, and policymakers. The completed report will be submitted to the Ethiopian Medical Laboratory Association, the Ethiopian Ministry of Health, and the Amhara Public Health Institutes, with a copy provided to Tana Flora Floriculture Industry. Additionally, the results will be published in a peer-reviewed journal and disseminated through local and international scientific seminars, conferences, and workshops.

6. Work flow

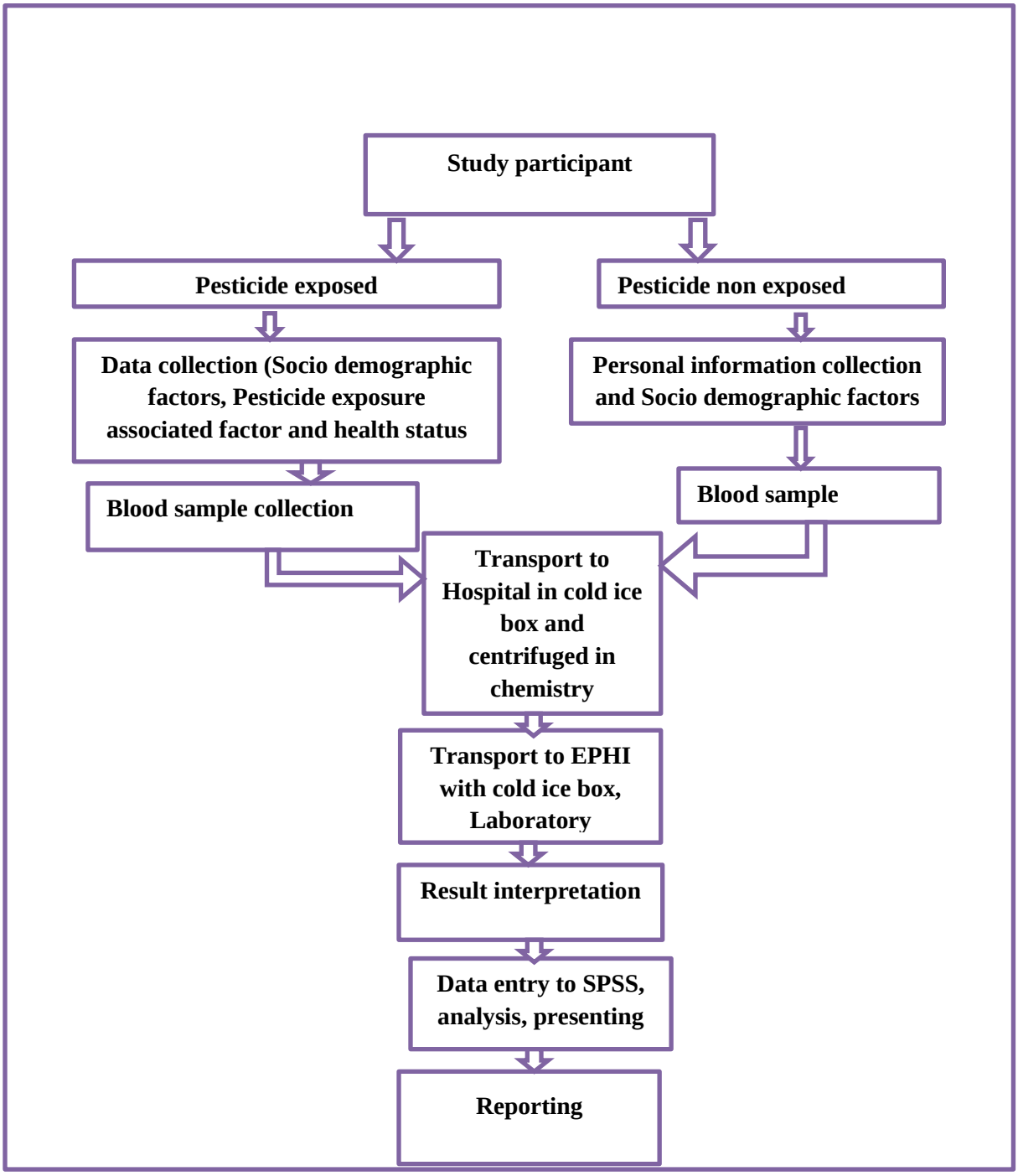


Figure 8: Work flow of the study on assessment of liver function and renal function among horticultural workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

7. Results

7.1. Socio-demographic Characteristics

A total of 102 study participants were included from 104 participants in the study, with a response rate of 98%, with 51 pesticide-exposed participants and 51 controls. There was no significant difference in sex, age categories, and marital status between case and control ($p > 0.05$). However, significant differences were observed in educational status, monthly income, and residency ($p < 0.001$). A greater proportion of controls had attained secondary education or higher, resided in urban areas, and earned more than 5000 ETB monthly (Table 5).

Table 5: Sociodemographic characteristics of the study participants on assessment of liver and renal function tests among horticultural workers at Tana flora Floriculture industry, Bahirdar, Ethiopia, 2025.

Sociodemographic characters	**Category**	**Group**		**Total (N=102) No. (%)**	**P-Value**
		Case (N=51) No. (%)	Control (N=51 No. (%)		
Sex	Male	15(29.4)	18(35.3)	33(32.4)	0.525[a]
	Female	36(70.6)	33(64.7)	69(67.6)	
Age	Mean ± SD	24.37± 2.75	24.2 ±2.80	/	0.201[b]
	18- 25	33(64.7)	34(66.7)	67(65.7)	0.835[a]
	26-32	18(35.3)	17(33.3)	35(34.3)	
Marital status	Married	52(50.5)	25(49)	77(50)	0.212[a]
	Single	42(40.8)	26(51)	68(44.2)	
	Windowed	1(1)	/	1(0.6)	
	Divorced	8(7.8)	/	8(5.2)	
Level of education	Illiterate	21(41.2)	3(5.9)	24(23.5)	<0.001[a]
	Reading and Writing	5(9.8)	8(15.7)	13(12.7)	
	Elementary	14(27.5)	11(21.6)	25(24.5)	
	Secondary school	7(13.7)	17(33.3)	24(23.5)	

	Diploma/ university above	4(7.8)	12(23.5)	16(15.7)	
Monthly income(ETB)	1500-3000	25(49)	4(7.8)	29(28.4)	<0.001[a]
	3000-5000	22(43.2)	21(41.2)	43(42.2)	
	>5000	4(7.8)	26(51)	30(29.4)	
Residency	Urban	9(17.6)	36(70.6)	45(44.1)	<0.001[a]
	Rural	42(82.4)	15(29.4)	57(55.9)	
BMI	<18.5 (Underweight)	3(5.9)	9(17.6)	12(11.8)	0.118[a]
	18.5-24.9 (Normal)	47(92.1)	42(82.4)	89(87.2)	
	25-30 (Overweight)	1(2)	0(0)	1(1)	

Notes: a- Pearson -Chi square, b- Independent sample t test, BMI-Body Mass index, ETB-Ethiopian Birr, SD- Standard Deviation

7.2. Occupational History, working environment and health perceptions among pesticide-exposed floriculture Workers

In this study, 17 (33.3 %) had worked in horticulture for less than 5 years, while 25 (49%) had 5–10 years of experience, and 9 (17.1%) had worked for more than 10 years (Table 6). Common symptoms reported during work included sweating 37(72.5%), skin rash 40(78.4%), cough 34 (66.7%), eye irritation 31 (60.8%), and headache 29 (56.7%) and others (Fatigue, back pain and diarrhea) 15(29.4%) (Figure 9). About 5.9 % reported experiencing foot edema, all of which occurred after employment. Health problems reported during the survey were skin problems 42 (82.4%), respiratory problems 39 (76.5%), neurological symptoms 25(49%), gastrointestinal problems 24 (47.1%), eye problems 26 (51%), reproductive problems 11 (21.6 %), and other problems (Musculoskeletal, weakness and urinary problems) 7 (13.7%) of participants (Figure 10)

Table 6: Occupational History, Working environment and Perceptions among floriculture workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

Variables	Categories	Frequencies	Percentages
Work experience	< 5years	17	33.3
	5-10 years	25	49
	>10 years	9	17.1
Mean work experience (years)	Mean ± SD	6.69 ± 3.14	/
Do you have foot edema?	Yes	3	5.9
	No	48	94.1
If your answer is yes, was the foot edema present before you were employed in this company? (N=3)	Yes	0	0
	No(Occurred after employment)	3	100
Current health perception	Excellent	2	3.9
	Very good	11	21.6
	Fear	19	37.3
	Poor	13	25.5
	Very poor	6	11.8
Working environment	Green house	32	62.7
	Packing	13	25.5
	Spraying	4	7.8
	Irrigation	2	3.9

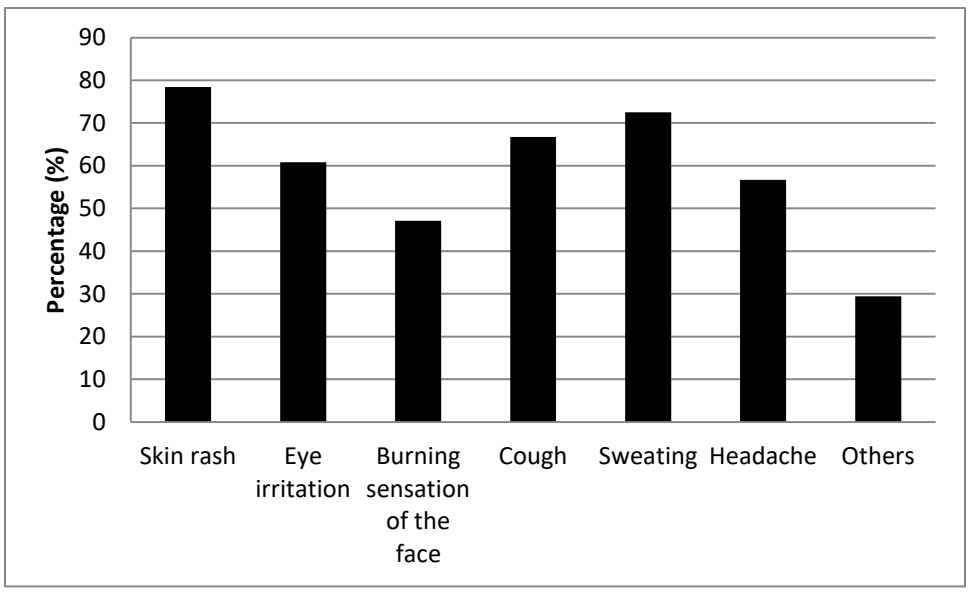


Figure 9: Pesticide-induced symptoms among horticultural workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025

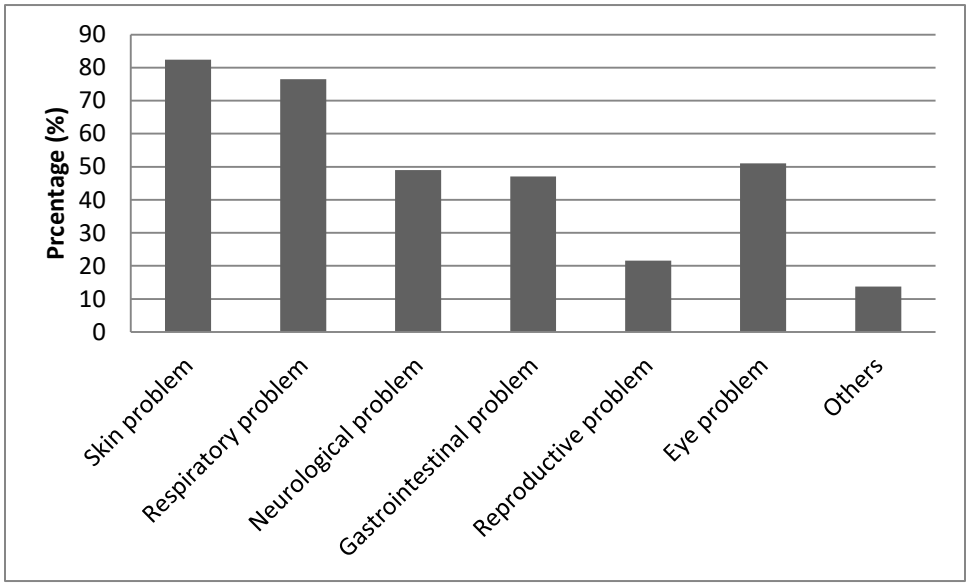


Figure 10: Pesticide-induced health problems among horticultural workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

7.3. Knowledge, Attitude and Practice of study participants among floriculture workers at Tana flora floriculture industry.

Only 25.5% of floriculture industry workers read and understand pesticide labels and know pesticides by name. Over half (52.9%) are aware of pesticide-related health problems and (54.9%) exposure routes. Overall, 51% of workers demonstrated good knowledge about pesticides. A majority either disagreed or strongly disagreed that pesticides have adverse effects on health (84.3%), indicating low risk perception. The overall attitude score was 51% positive, 49% negative. Only 35.3% reported using PPE, and 41.2% changed clothes before going home. However, 78.4% washed hands after work, and 66.7% had good overall

practices. After pesticide exposure, the majorities go to health facility (58.8%), 29.4% stopped work and rested, and 11.8% used traditional medicine (Table 7).

Table 7: Knowledge, attitude and practice of study participants among floriculture workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

A. Knowledge			
Variables	Category	Frequency	Percentage (%)
Do you read and understand pesticide labels	Yes	13	25.5
	No	38	74.5
Do you know pesticides by name?	Yes	13	25.5
	No	38	74.5
Do you know pesticide-related health problems?	Yes	27	52.9
	No	24	47.1
Do you know routes of exposure?	Yes	28	54.9
	No	23	45.1
If yes, what are the routes of exposure? [a]	Skin contact	28	54.9
	Ingestion	28	54.9
	Eye contact	28	54.9
	Inhalation	27	52.9
Overall knowledge	**Good**	**26**	**51**
	Poor	**25**	**49**
B. Attitude			
Do you think all pesticides have adverse effects on human health?	Strongly disagree	18	35.3
	Dis agree	25	49
	Agree	3	5.9
	Strongly agree	5	9.8
Do you think pesticides should be discouraged?	Strongly disagree	19	37.3
	Dis agree	23	45.1
	Agree	4	7.8
	Strongly agree	5	9.8
Do you think PPE prevents pesticide exposure?	Strongly disagree	19	37.3
	Dis agree	23	45.1
	Agree	4	7.8
	Strongly agree	5	9.8
Do you think PPE is important?	Strongly disagree	19	37.3
	Dis agree	23	45.1
	Agree	4	7.8
	Strongly agree	5	9.8

Do you think, good pesticide handling reduces health problems?	Strongly disagree	19	37.3
	Dis agree	23	45.1
	Agree	4	7.8
	Strongly agree	5	9.8
Overall attitude	**Positive**	**26**	**51**
	Negative	**25**	**49**
C. Practice			
Do you use personal protective equipment?	Yes	18	35.3
	No	33	64.7
Do you take a bath after pesticides are applied?	Yes	7	86.3
	No	44	13.7
Do you wash your hands immediately after work?	Yes	40	78.4
	No	11	21.6
Do you change clothes before going home?	Yes	21	41.2
	No	30	58.8
Do you consume coffee?	Yes	27	52.9
	No	24	47.1
Do you consume alcohol?	Yes	16	31.4
	No	35	68.6
Do you have the habit of eating and drinking, in the working Area?	Yes	15	29.4
	No	36	70.6
What action to be taken after pesticide exposure?	Traditional Medicine	6	11.8
	Stop work and rest	15	29.4
	Go to the health facility	30	58.8
Overall practice	**Good**	**34**	**66.7**
	Poor	**17**	**33.3**

Note: a-Multi-response questions

7.4. Environmental and institutional factors of floriculture workers at Tana Flora Floriculture Industry

Among the study participants, only 25.5 % and 23.5% took periodical checkup and on job training, respectively. About 60.8 % of the study participant, had access to (PPE). A majority, 70.6%) of study participants disposed of empty pesticide containers by burning, while others buried them, 17.6%, or threw them in the field, 11.8%. Additionally, 54.9 % indicated the absence of safe pesticide storage at their workplace (Table 8).

Table 8: Environmental and institutional factors of floriculture workers at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

Variables	Category	Frequency	Percentiles (%)
Is there safe pesticide storage in the working area?	Yes	23	45.1
	No	28	54.9
What types of methods do you use to eliminate or dispose of empty pesticide containers?	Burn	36	70.6
	Bury in the ground	9	17.6
	Through the field	6	11.8
Do you take training before work (pre-training)?	Yes	10	19.6
	No	41	80.4
Do you take a periodic check-up?	Yes	13	25.5
	No	38	74.5
Do you have a supply of personal protective equipment?	Yes	31	60.8
	No	20	39.2
Do you take on job training?	Yes	12	23.5
	No	39	76.5

7.5. Comparison of liver, renal function, and butyrylcholinesterase tests among pesticide-exposed and controls

The comparison of LFTs, RFTs and BChE between pesticide-exposed workers and unexposed controls showed statistically significant differences in several parameters. ALT was significantly higher in cases (27.29 ± 3.30 U/L) compared to control (22.91 ± 1.72 U/L), $p < 0.001$, and AST also higher in cases (27.99 ± 5.23 U/L) compared to controls (20.37 ± 2.81 U/L, $p < 0.001$. TBL (0.87 ± 0.22 vs. 0.37 ± 0.14 mg/dL, $p < 0.001$) and DBL (0.28 ± 0.10 vs. 0.15 ± 0.06 mg/dL, $p < 0.001$) were higher in cases; whereas albumin was lower (4.37 ± 0.40 vs. 4.64 ± 0.26 mg/dL, $p < 0.001$). Urea was significantly higher in case (40.35 ± 6.40) compared to controls (20.73 ± 1.80 mg/dL), $p <0.001$, creatinine also significantly higher in cases (0.92 ± 0.12) than controls (0.59 ±

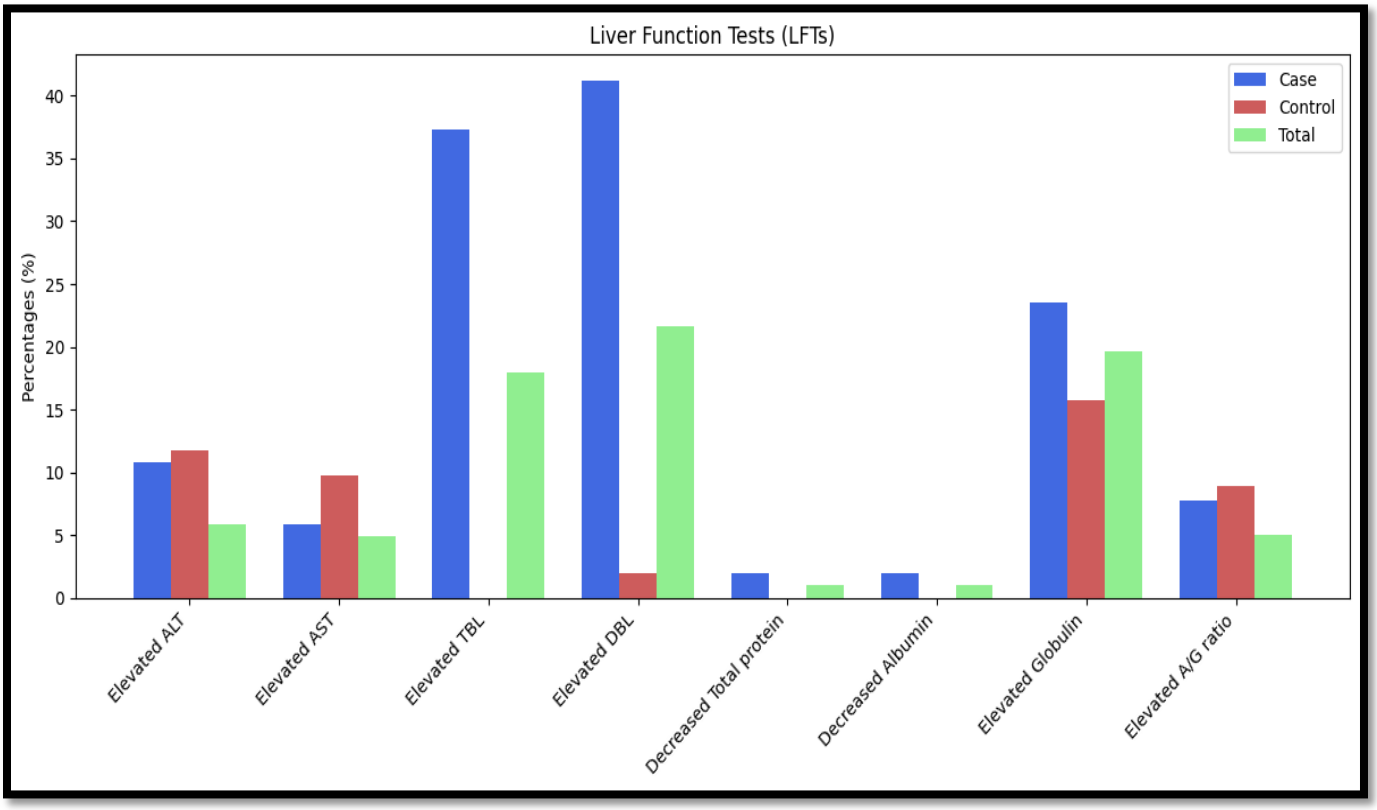
0.08 mg/dL), P <0.001. In contrast, eGFR was lower in cases (98.84 ± 18.03 mL/min/1.73 m²,) than controls (132.54 ± 6.86), p < 0.001). BChE activity was also significantly reduced in cases (5285.22 ± 1013.29) than controls (6579.00 ± 1159.73 U/L,) p < 0.001) (Table 9).

Table 9: Comparison of liver function tests among pesticide-exposed and control study participants at Tana Flora, Floriculture, Bahirdar, Ethiopia, 2025.

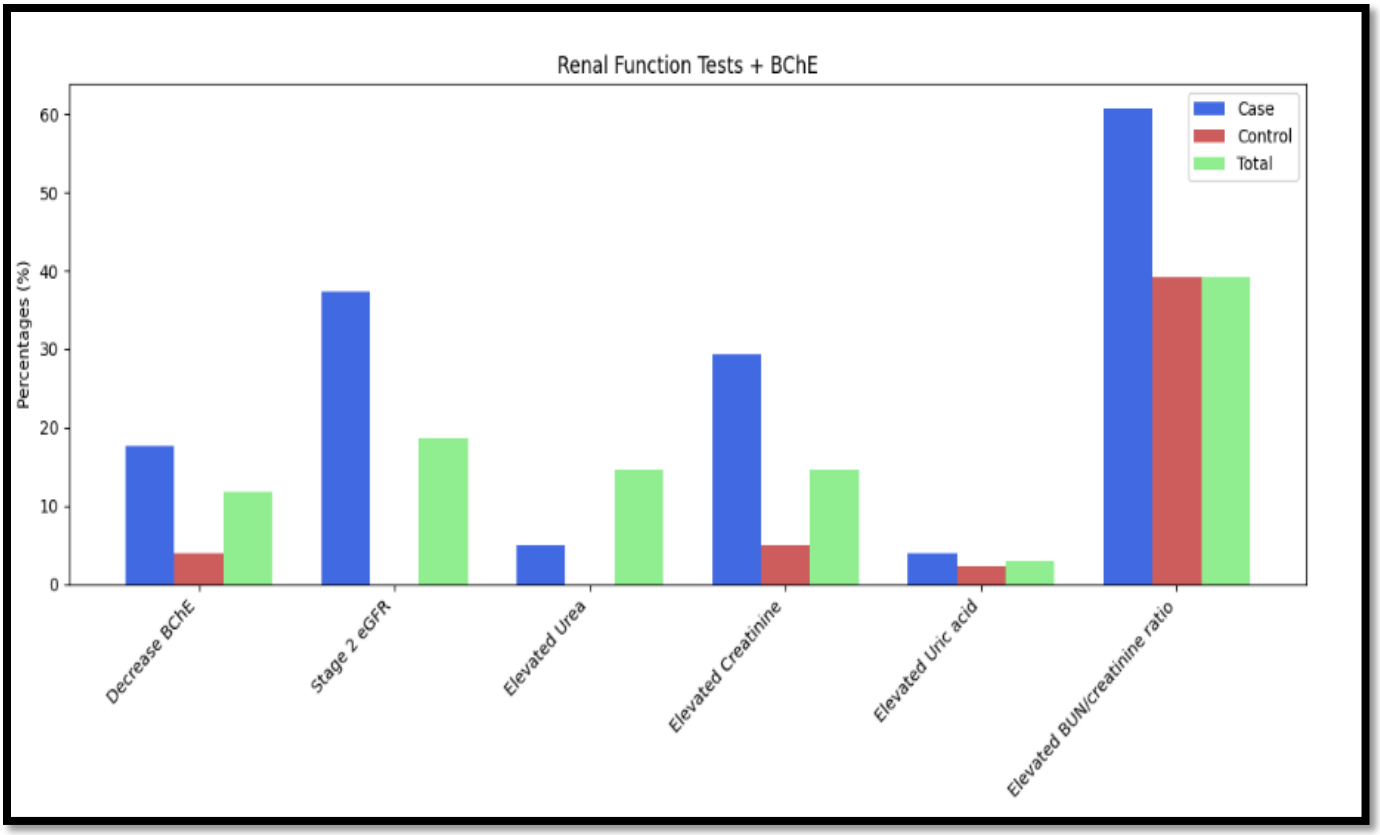
Parameter	Case (N=51)		Control (N=51)		Mean Difference	p-value
	(Mean ± SD)	(Min-Max)	(Mean ± SD)	(Min–Max)		
ALT(U/L)	27.29 ± 3.30	21.40–34.50	22.91 ± 1.72	19.50–26.60	4.38	**< 0.001**
AST(U/L)	27.99 ± 5.23	15.90–41.50	20.37 ± 2.81	15.20–27.20	7.62	**< 0.001**
ALP(U/L)	78.27 ± 6.51	63.00–89.10	78.02 ± 5.59	66.00–88.00	0.25	0.752
TBL (mg/dL)	0.87 ± 0.22	0.51–1.32	0.37 ± 0.14	0.13–0.67	0.50	**< 0.001**
DBL mg/dL	0.28 ± 0.10	0.10–0.53	0.15 ± 0.06	0.04–0.30	0.13	**< 0.001**
TP (mg/dL)	7.52 ± 0.40	6.60–8.34	7.59 ± 0.36	6.89–8.32	−0.08	0.219
Albumin (mg/dL)	4.37 ± 0.40	3.34–5.08	4.64 ± 0.26	4.07–5.06	−0.27	**< 0.001**
Globulin (mg/dL)	3.14 ± 0.46	1.80–4.10	2.96 ± 0.41	2.31–4.05	0.18	**0.019**
A/G Ratio (mg/dL)	1.44 ± 0.34	0.86–2.67	1.60 ± 0.26	1.05–2.18	−0.16	**0.002**
Urea (mg/dL)	40.35 ± 6.40	28.20–54.40	20.73 ± 1.80	17.80–24.20	19.63	**< 0.001**
Creatinine (mg/dl)	0.92 ± 0.12	0.70–1.23	0.59 ± 0.08	0.40–0.78	0.33	**< 0.001**
Uric Acid (mg/dl)	4.53 ± 0.76	3.00–6.30	4.45 ± 0.76	3.01–5.80	0.08	0.478
BUN/Creatinine Ratio	20.68 ± 3.00	13.73–26.44	16.86 ± 3.04	11.96–24.30	3.82	**< 0.001**
eGFR (CKD-EPI 2021)	98.84 ± 18.03	64.52–130.74	132.54 ± 6.86	110.45–145.61	−33.70	**< 0.001**
BChE (U/L)	5285.22 ±1013.29	3195–7281	6579.00 ±1159.73	4484–8697	−1293.78	**< 0.001**

7.6. Comparison of abnormal results of liver function, renal function, and butyrylcholinesterase tests among pesticide-exposed and controls

Among the study participants, the prevalence of low BChE was significantly higher in cases (9/51, 17.6%) compared with controls (2/51, 3.9%), p =0.025. In LFTs, elevated ALT was observed in 6/51 cases (11.8%) and none of the controls (p = 0.027). TBL and DBL were significantly higher in cases (TBL high: 19/51, 37.3%; DBL high: 21/51, 41.2%) compared with controls (0 and 1/51, respectively), with p < 0.001. For renal function, reduced eGFR (Stage 2) was found in 19/51 cases (37.3%) versus none of the controls, p < 0.001. Elevated creatinine occurred in 15/51 cases (29.4%) and 0 controls (p < .001), while abnormal BUN/creatinine ratio was more frequent in cases (31/51, 60.8%) compared with controls (9/51, 17.6%), p < 0.001(Figure11).



A.



B.

Figure 11: A: Comparison of abnormal results of liver function tests; B: Comparison of abnormal renal function, and Butrylcholinesterase among pesticide-exposed and controls at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025

7.7. Effect of Work duration on liver, renal function tests and butyrylcholinesterase of pesticide exposed workers

A Kruskual Wallis test with Bonferroni post hoc comparison performed among floriculture workers showed that longer occupational exposure (>10 years) demonstrated, significantly higher median levels of ALT ($p < 0.05$), DBL and TBL ($p < 0.05$ for both), and creatinine ($p < 0.05$) compared to < 5 years and 5-10 years' work experience greater than 10 years' work experience had significantly higher AST($p<0.05$), urea ($p<0.05$) and uric acid ($p<0.05$) compared to 5-10 years. In contrast, albumin ($p < 0.05$) and the A/G ratio ($p < 0.05$) were

significantly lower with workers >10 years work experience compared with <5 years and 5-10 years of experience. In addition, eGFR decreased progressively with increasing years of service ($p < 0.05$), >10 years compared with <5 years. BChE activity also showed a decreasing trend with longer exposure, although the difference was not statistically significant ($p > 0.05$) (Table 10).

Table 10: Effect of Work duration on liver, renal function tests and butyrylcholinesterase among floriculture workers, Bahirdar, Ethiopia, 2025.

Parameters	**<5 years (Median ± IQR) (N=17)**	**5–10 years (Median ± IQR, (N=25)**	**>10 years (Median ± IQR) (N=9)**
ALT	25.8 ± 2.65	25.9 ± 4.30	31.1 ± 5.10 **a,b**
AST	27.3 ± 5.85	26.0 ± 3.70	32.9 ± 7.95 **b**
ALP	77.0 ± 8.85	80.0 ± 10.35	82.0 ± 4.55
TBL	0.725 ± 0.34	0.880 ± 0.21	1.180 ± 0.12 **a,b**
DBL	0.267 ± 0.129	0.270 ± 0.156	0.380 ± 0.111 **a,b**
Total Protein	7.26 ± 0.55	7.59 ± 0.52	7.56 ± 0.46 **c**
Albumin	4.53 ± 0.48	4.56 ± 0.44	3.87 ± 0.25 **a,b**
Globulin	3.00 ± 0.37	3.13 ± 0.59	3.65 ± 0.53 **a**
A/G Ratio	1.54 ± 0.22	1.41 ± 0.33	1.10 ± 0.16 **a,b**
Urea	41.8 ± 7.27	36.2 ± 9.40	47.7 ± 9.30 **b**
Creatinine	0.85 ± 0.14	0.88 ± 0.12	1.07 ± 0.07 **a,b**
Uric Acid	4.80 ± 1.16	4.10 ± 1.05	5.10 ± 0.64 **b**
BUN/Creatinine Ratio	21.55 ± 3.13	19.56 ± 4.97	20.64 ± 4.24 **c**
eGFR (CKD-EPI 2021)	106.51 ± 33.27	96.09 ± 28.55	80.15 ± 26.19 **a**
BChE	5559 ± 1873	5487 ± 995	4761 ± 1992

Note: Data are presented as median ± interquartile range (IQR). Independent-sample Kruskual-Wallis test was used to compare groups. Significant pairwise comparisons were determined using Bonferroni-adjusted post-hoc tests.

- **a-** adjusted P. value <0.05 when >10 years compared with <5 years
- **b-** adjusted P. value <0.05 when >10 years compared with 5-10 years
- **c-** adjusted P. value <0.05 when 5-10 years compared with <5 years

7.8. Factors associated with liver function, renal function, and butyrylcholinesterase among pesticide-exposed horticultural workers

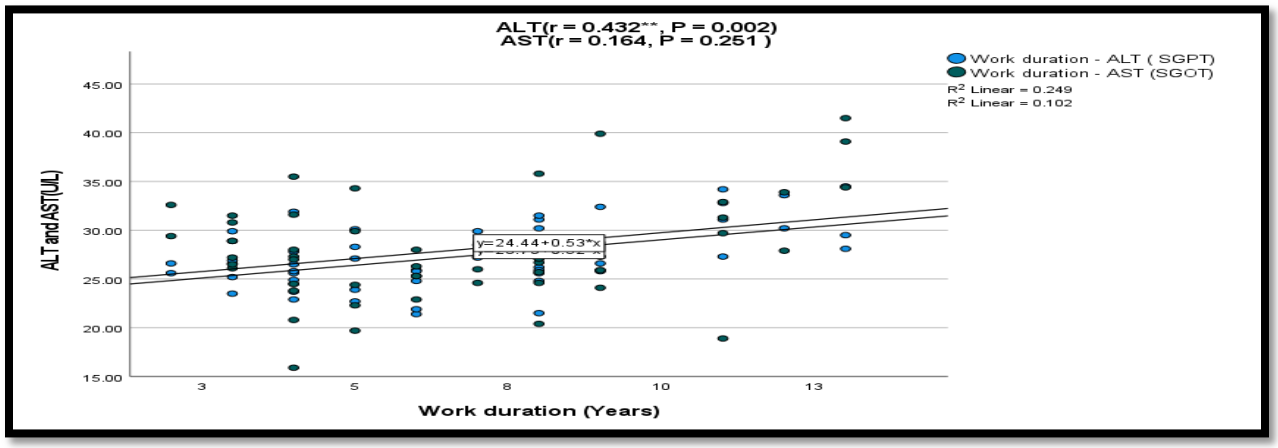
7.8.1. Correlation of associated factors with liver function tests, renal function tests, and butyrylcholinesterase.

Age showed significant positive correlations with several LFTs and RFTs : ALT (r = 0.430, p = 0.002), AST (r = 0.303, p = 0.031), ALP (r = 0.303, p = 0.031), TBL (r = 0.337, p = 0.016), DBL (r = 0.378, p = 0.006), urea (r = 0.287, p=0.041) ,Creatinine (r = 0.614, p = <0.001) and a significant negative correlation with albumin (r = -0.322, p = 0.021), A/G ratio (r = -0.341, p = 0.014) and eGFR(r = -0.408, p =0.003). Sex (female) was significantly negatively correlated with AST (r = -0.420, p = 0.002), eGFR (r = -0.541, p < 0.001), and BChE (r = -0.374, p = 0.007). BMI had positive correlations with ALT (r = 0.301, p = 0.032), TBL (rho = 0.412, p = 0.003), DBL (r= 0.382, p = 0.006), creatinine (r = 0.462, p < 0.001), and uric acid (r = 0.284, p = 0.043), while eGFR correlated negatively (r = -0.444, p = 0.001). PPE use was positively correlated with BChE (r = 0.301, p = 0.032. Work duration showed significant positive correlations with ALT (r = 0.432, p = 0.002), TBL (r = 0.431, p = 0.002), DBL (r = 0.309, p = 0.027), globulin (r = 0.402, p = 0.003), and a significant negative correlation with albumin (r = -0.284, p = 0.043) and A/G ratio (r = -0.426, p = 0.002) (Table 11).

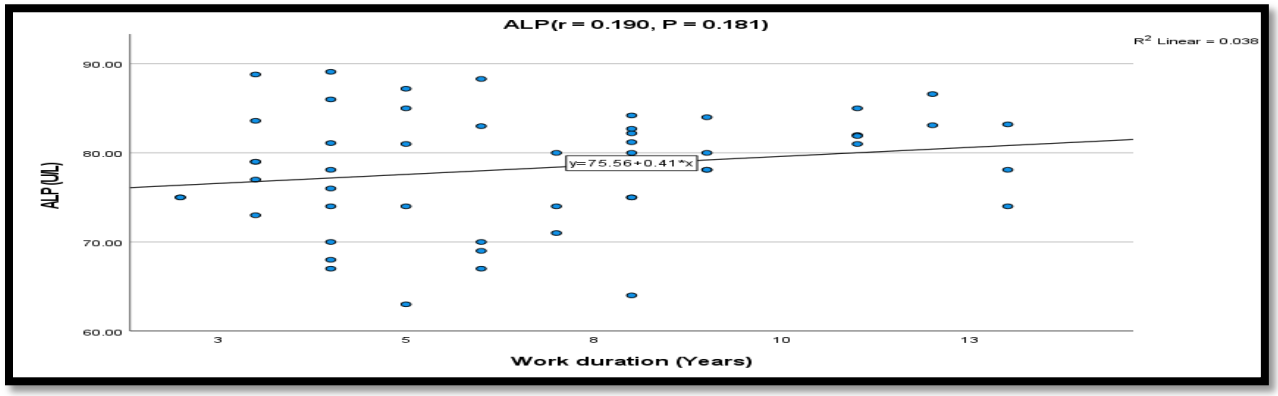
Table 11: Spearman correlation of liver function, renal function, and Butrylcholinesterase with associated factors among horticultural workers at Tana Flora floriculture industry, Bahirdar, Ethiopia.

Parameters	Associated factors									
	Age(years)		Sex(Female)		BMI(Kg/M^2)		PPE use (Yes)		Work duration (Years)	
LFT	**R**	**P. value**	**R**	**P.value**	**R**	**P.value**	**r**	**P.value**	**R**	**P.value**
ALT	.430**	.002	-.089	.534	.301*	.032	-.001	.992	.432**	.002
AST	.303*	.031	-.420**	.002	.209	.142	-.035	.808	.164	.251
ALP	.303*	.031	.072	.617	.314*	.276	.201	.141	.190	.181
TBL	.337*	.016	.064	.654	.412**	.003	0.082	.566	.431**	.002
DBL	.378**	.006	-.064	.654	.382**	.006	.000	1.000	.309*	.027
TP	-.103	.472	.054	.706	0.044	.760	.033	.816	.125	.382
Albumin	-.322*.	.021	0.029	.839	-.070	.626	.028	.846	-.284**	.0043
Globulin	.245	.084	-0.001	.992	.129	.369	-.0039	.786	.402**	.003
A/G ratio	-.341*	.014	-.012	.935	-.132	.356	.000	1.000	-.426**	.002
RFT	**R**	**P.value**	**R**	**P.value**	**R**	**P.value**	**r**	**P.value**	**r**	**P.value**
Urea	.287*	.041	-.064	.654	.252	.075	-.169	.237	.195	.169
Creatinine	.614**	<.001	-.073	.610	.462**	<.001	.130	.364	.512**	<.001
Uric acid	.197	.167	-.199	.161	.284*	.043	-.204	.152	.087	.546
BUN /Creatinine ratio	-.238	.483	.099	.488	-.100	.483	-.220	.120	.255	.072
eGFR	-.408**	.003	-.541**	<.001	-.444**	.001	-.166	.245	-.365**	.009
BChE	-.084	.559	-.374**	.007	-.286*	.042	.301*	.032	-.198	.163

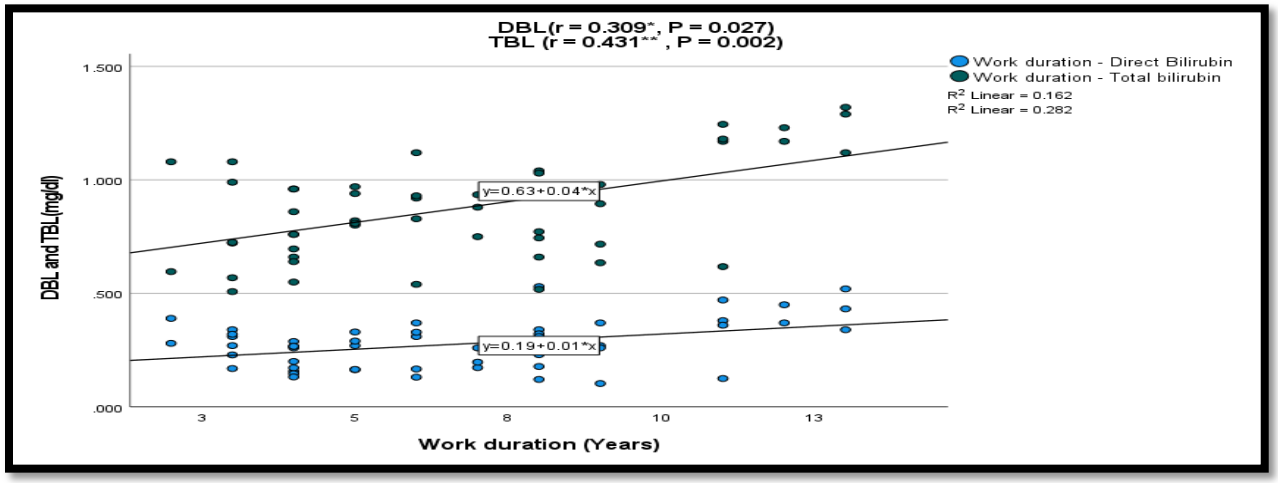
Notes: r- spearman correlation coefficient
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).



(A)



(B)



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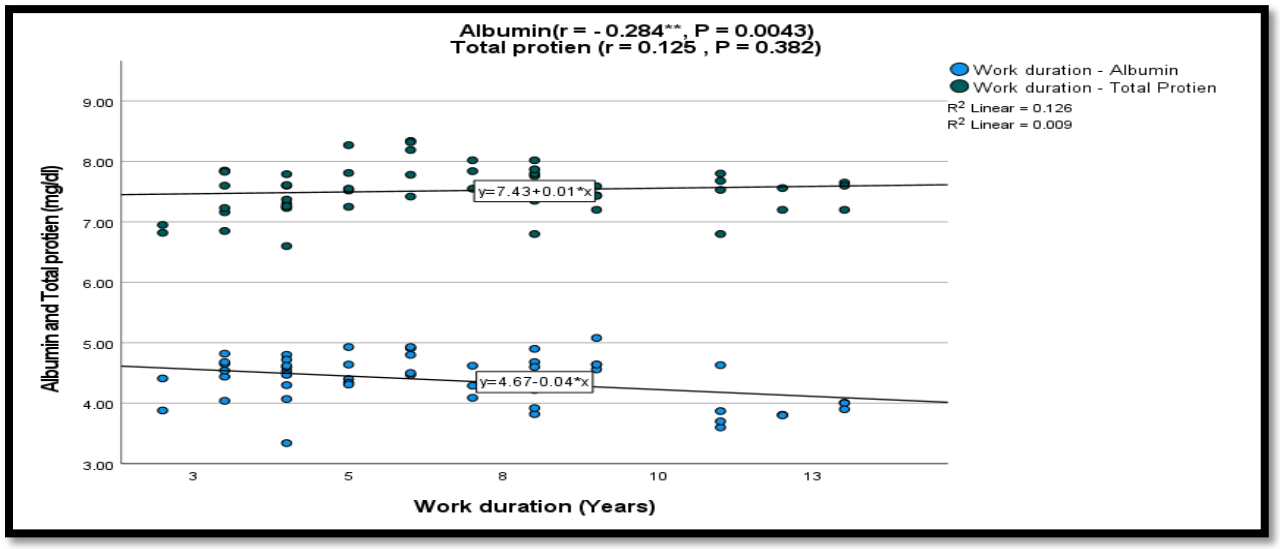
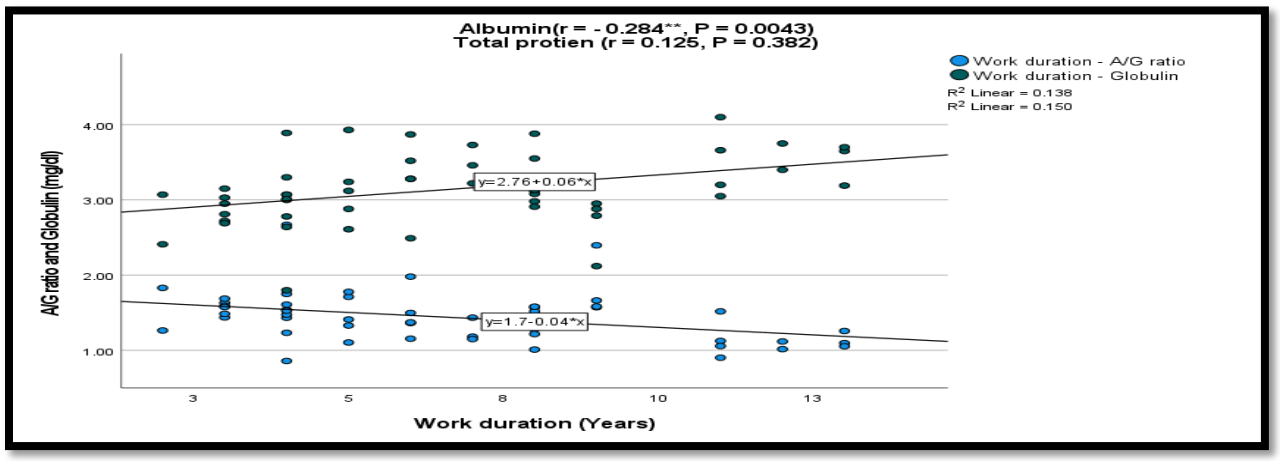
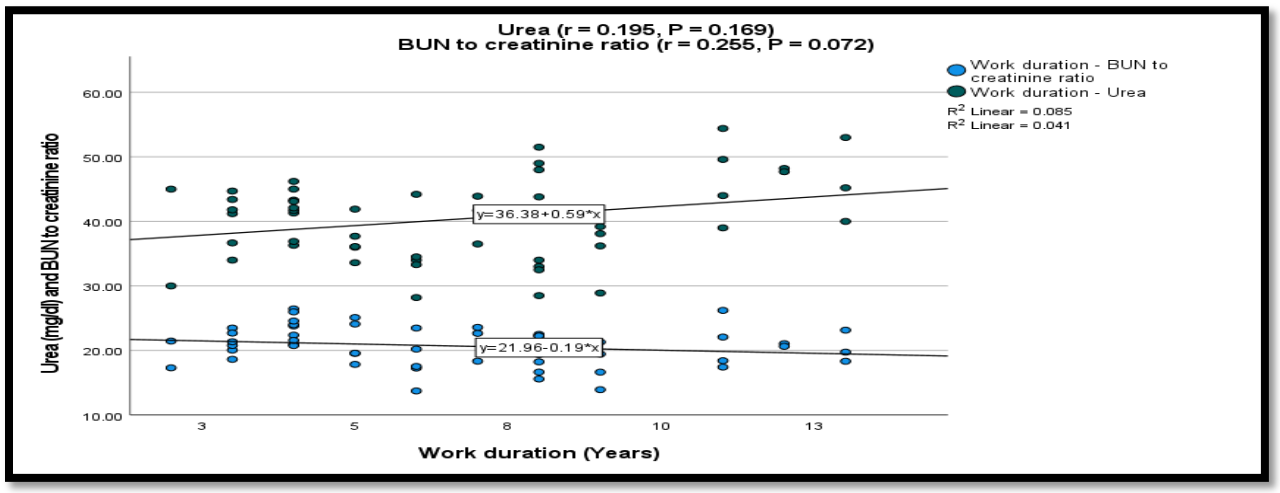

Albumin(r = - 0.284**, P = 0.0043)
Total protien (r = 0.125 , P = 0.382)
Work duration - Albumin
Work duration - Total Protien
R² Linear = 0.126
R² Linear = 0.009
y=7.43+0.01*x
y=4.67-0.04*x
Albumin and Total protien (mg/dl)
Work duration (Years)

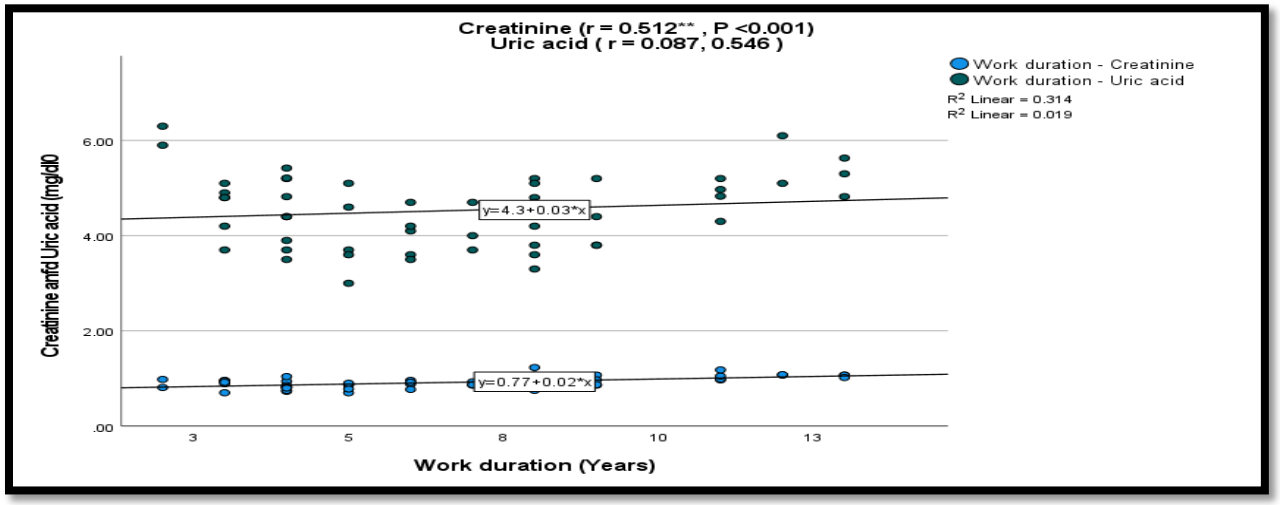
(D


Albumin(r = - 0.284**, P = 0.0043)
Total protien (r = 0.125, P = 0.382)
Work duration - A/G ratio
Work duration - Globulin
R² Linear = 0.138
R² Linear = 0.150
y=2.76+0.06*x
y=1.7-0.04*x
A/G ratio and Globulin (mg/dl)
Work duration (Years)

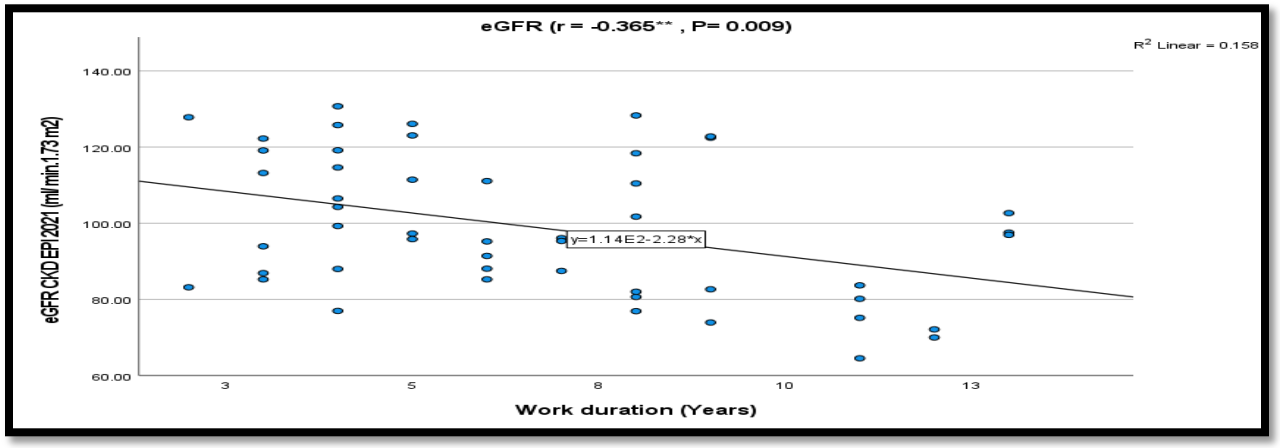
(E)


Urea (r = 0.195, P = 0.169)
BUN to creatinine ratio (r = 0.255, P = 0.072)
Work duration - BUN to creatinine ratio
Work duration - Urea
R² Linear = 0.085
R² Linear = 0.041
y=36.38+0.59*x
y=21.96-0.19*x
Urea (mg/dl) and BUN to creatinine ratio
Work duration (Years)

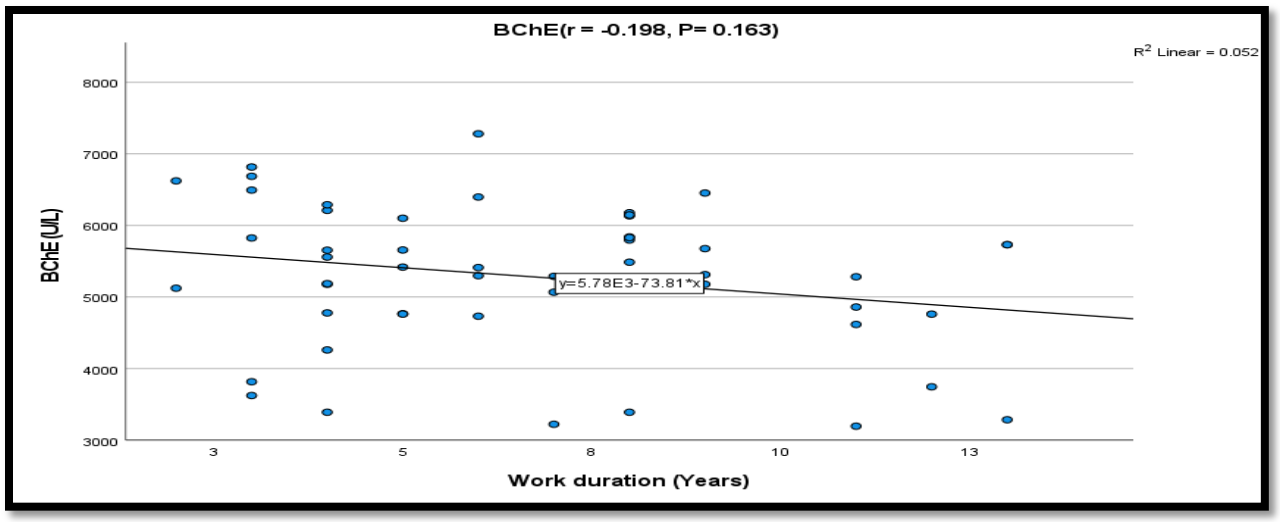
(F)


Creatinine (r = 0.512** , P <0.001)
Uric acid (r = 0.087, 0.546)
Work duration - Creatinine
Work duration - Uric acid
R² Linear = 0.314
R² Linear = 0.019
Creatinine amfd Uric acid (mg/dl0
6.00
4.00
2.00
.00
y=4.3+0.03*x
y=0.77+0.02*x
3
5
8
10
13
Work duration (Years)

(G)


eGFR (r = -0.365** , P= 0.009)
R² Linear = 0.158
eGFR CKD EPI 2021 (ml/min.1.73 m2)
140.00
120.00
100.00
80.00
60.00
y=1.14E2-2.28*x
3
5
8
10
13
Work duration (Years)

(H)



(I)

Figure 12: Scatter plot of work duration and; (A): ALT and AST; (B): ALP; (C): TBL and DBL; (D): Albumin and Total protein; (E): Globulin and Albumin to globulin ratio; (F): Urea and BUN to creatinine ratio; (G); Creatinine and Uric acid; (H): eGFR; (I): BChE

7.8.2. Stepwise multiple linear regression between associated factors and biochemical profiles

Stepwise multiple linear regression between associated factors and biochemical profiles among floriculture workers showed that BChE levels were higher among workers who used PPE (β = 724.28, p = 0.005) and took regular medical checkups (β = 936.03, p = 0.020). In contrast, lower in females (β = –697.99, p = 0.009) and those with higher BMI (β = –203.64, p = 0.002). Work duration was a strongly associated with increase ALT (β = 0.524, p < 0.001) and AST (β = 0.433, p = 0.039). AST levels were significantly lower in females (β = –5.00, p < 0.001). ALP increased with age (β = 0.725, p = 0.029), and increase work duration also significantly associated with increased TBL (β = 0.037, p < 0.001)). Albumin decreased with age (β = –0.053, p = 0.008), while globulin increased with work duration (β = 0.057, p = 0.005). BMI significantly associated with increase urea (β = 1.544, p = 0.012) and creatinine (β = 0.024, p <

0.001), whereas eGFR was lower among females (β = –23.369, p < 0.001) and declined with increasing age (β = –3.059, p < 0.001) and BMI (β = –2.956, p = 0.014) (Table 12).

Table 12: Stepwise multiple linear regression of biochemical parameters and associated factors Among pesticide-exposed participants (N=51).

Parameters	**Adjusted R^2**	**Predictors**	**B**	**P. value**	**ANOVA**
					F(P.value)
BChE (IU/L)	0.340	PPE use(1)	724.28	0.005	7.438(<0.001)
		Sex(1)	-697.99	0.009	
		BMI(kg/m^2)	-203.64	0.002	
		Periodical check-up(1)	936.03	0.020	
ALT(IU/L)	0.234	Duration of work(years)	0.524	<0.001	16.264(<0.001)
AST(IU/L)	0.262	Sex(1)	-5.00	<0.001	9.883(<0.001)
		Work duration(years)	0.433	0.039	
ALP(IU/L)	0.075	Age	0.725	0.029	5.062(0.029)
TBL (mg/dl)	0.267	Work duration(years)	0.037	<0.001	19.223(<0.001)
DBL (mg/dl)	0.286	Age	0.014	0.005	11.007(<0.001)
		BMI(kg/m^2)	0.026	0.004	
Total protein(mg/dl)	-	-	-	NS	-
Albumin(mg/dl)	0.116	Age (years)	-0.053	0.008	7.561(0.008)
Globulin (mg/dl)	0.132	Work duration (years)	0.057	0.005	8.623(0.005)
A/G ratio	0.121	Work duration(years)	-0.040	0.007	7.852(0.007)
Urea (mg/dl)	0.105	BMI (kg/M^2)	1.544	0.012	6.895 (0.012)
Creatinine (mg/dl)	0.409	BMI (kg/m^2)	0.024	<0.001	18.315(<0.001)
		Age(years)	0.022	0.020	

Uric acid (mg/dl)	-	-	-	NS	-
BUN/Creatinine ratio	0.059	Take bath after pesticide applied(1)	-2.407	0.048	4.129(0.048)
eGFR-CKD-EPI-2021(ml/min/1.72m^2)	0.584	Sex(1)	-23.369	<0.001	24.419(<0.001)
		Age(years)	-3.059	<0.001	
		BMI (kg/m^2)	-2.956	0.014	
Note: Sex (0: Male; 1:Female), PPE- personal protective equipment(0:No;1:Yes), Periodical check-up(0:No;1:Yes), Take bath after pesticide applied (0: No;1:Yes),NS- Not significant					

8. Discussion

The present study aimed to assess LFT, RFT, and BChE levels among floriculture workers in Ethiopia. The study consisted of 102 participants, 51from pesticide-exposed floriculture workers and 51 non-pesticide-exposed participants. During the study, it was found that the floriculture industry used approximately 34 different pesticides. Of these, 18 (53%) were classified as unlikely to present acute hazard (Class U), 13 (38%) fell under Class II (moderately hazardous), 2 (6%) were in Class Ib (highly hazardous), and 1 (3%) belonged to Class III (slightly hazardous) according to the WHO classification of pesticides by hazard (62).

In our study, 34(66.1%) had worked in the floriculture industry for 5 years or more, suggesting long-term exposure to pesticides. The mean duration of work was 6.69 ± 3.14 years, which points to a chronic exposure risk. Floriculture workers often absorb pesticides through skin contact, inhalation, or ingestion. These chemicals travel through the bloodstream and can affect various organs, especially the liver and kidneys, which work to break down and remove toxins. Over time, this exposure can lead to serious damage to both organs (6, 81).

During the study, the most commonly reported symptoms among pesticide-exposed floriculture workers included excessive sweating, skin rash, cough, eye irritation, and headache. Different literatures reported similar findings (43, 77, 81, 132-134).

Many different health problems were reported in this study, with predominantly skin problems, respiratory problems, neurological problems, gastrointestinal problems, and eye problems. These kinds of problems have also been seen in other studies and are often associated with low use of personal protective equipment, poor practices of pesticide use, and low awareness about pesticide exposure. Pesticide exposure at work does not just cause immediate health problems; it can also lead to subtle changes in the body that might have unknown long-term effects (135, 136). In our study, participants were exposed to mixed pesticides. The combination of two or more chemicals can have synergistic effects, which may increase their toxicity and potentially harm various organ systems in the body (137).

About 16 (47%) of the pesticides in the study area belongs to moderately hazardous and highly hazardous categories and poses potential chronic and acute health risks to floriculture workers. The most commonly used pesticide groups were insecticides (organophosphates, spinosyns, and neonicotinoids) and fungicides (morpholines and diazoles); Miticides and herbicides were less frequent. 6 (17.6%) of the pesticides constitute organophosphates, and 3 (8.8%) are carbamates found in the pesticides in the study this finding supported by the study (53, 138, 139). Organophosphates and carbamates are known for their neurotoxic effects, inhibiting cholinesterase activity and potentially leading to acute poisoning or long-term neurological impairment (140).

In our study, the level of knowledge among floriculture workers regarding pesticide use and associated health risks was generally low. About 26 study participants (51%; 95% CI: 36.78%, 65.18%) demonstrated good overall knowledge. The study was consistent with study, western Bhutan's (41%) had high and moderate knowledge (66), Indonesia (40.4%) had good knowledge (67), Gondar town, Ethiopia (36.2%) had good knowledge (69), southwest Shewa zone, Ethiopia (39.2%) had good knowledge (71). The study was lower than in Kelthan, Malaysia (68.7%) had high and moderate knowledge (64). In contrast, it was higher than a study conducted in Thailand, where 22.8% of participants were reported to have good knowledge regarding pesticide use (139), and Bahirdar, Ethiopia (33.3%) (70). The variation may be due to the study setting and sample size. In this study only 13(25.5%; 95% CI:13%, 38%) of study participants read and understood of pesticide labeling and knew pesticides by name this result is

consistent with in Kuwait (30%) (65), Bule Hora, Ethiopia (26%)(68), But higher than Bahirdar, Ethiopia (13.7%)(70). The difference may be due to a sample size difference. Knowledge about pesticide use is really important for floriculture workers it helps them understand the risks and take the right actions to protect their health and safety (65, 141). The results show that 28(54.9%; 95% CI: 41%, 69%) identified possible exposure routes, and 27(52.9%; 95% CI: 39%, 67%) were aware of problems with health associated with pesticides. This suggests that nearly half of the workers had a basic understanding of pesticide routes of entry and health problems which may be caused by the high rate of illiteracy (41.2%). In contrast, 76.3% of participants in a prior study in Bahirdar, Ethiopia, were aware of the exposure routes, and 85.3% were aware of health problems related with pesticides (70). The difference may be due to low illiteracy rate in the previous study which was only 12%.

In this study, 26(51%: 95% CI: 36.78%, 65.18%) of study participants had a positive attitude towards pesticide handling practices. The study was consistent with Kota Bharu, Kelantan, Malaysia (43.7%)(64), Bahirdar, Ethiopia (44.7%)(70), Thailand (51.5%) (63), Bhutan(63%) (66), and Gondar, Ethiopia (49.1%)(69). In contrast higher than study done in Indonesia (35%)(67). The difference may be due to differences in study setup and educational label differences. In this study, only 17.6% believed wearing PPE is important, 15.7% believed pesticides have adverse effects, 17.6% believed PPE is important to prevent pesticide exposure, and 17.6% believed good pesticide handling reduces health problems. The results were lower than a previous study done in Bahirdar, Ethiopia (59.3%) (70). The discrepancy may be due to the high illiteracy rate in our study setup.

In our study, 34 (66.7%; 95 % CI: 53.28%, 80.06%) of the study participants had good practice towards pesticide handling. The result was in line with previously done Bahirdar, Ethiopia (61.35%)(70). The result was higher than Thailand (6%)(63), Kelthan, Malaysia (21.5%)(64), Indonesia (31.6%)(67), and South west Shewa (26.6%)(71), Gondar, Ethiopia (36.2%)(69) . While lower than Bahirdar, Ethiopia (61.35%)(70). The differences may be due to educational level differences, study setup, and sample size differences. In this study 18 (35%; 95 % CI: 22%, 49%) use PPE during work. The result was in line with Kuwait (42%)(65), Bahirdar, Ethiopia (37.7%)(70), South west Shewa (29.9%) (71). The study is higher than Gondar, Ethiopia only 8.2% use PPE. In this study, 6(11.8%) of pesticide exposed participants seeking traditional

medicine after pesticide exposure has also been reported in a previous study conducted in Ethiopia (70). This indicates that workers had limited awareness of the harmful effects of pesticides. Early recognition of pesticide overexposure and earlier first-aid intervention are essential (142). In this study only 12(23.5%) had on job training the result is consistent with south west Shewa (28%) (71). The result indicated there is a need of periodical training to reduce pesticide exposure. 31 (60.8%) of the study participants get PPE from the company the study was consistent with Bahirdar (52.3%)(70). The results indicated there is shortage of PPE supply on floriculture industry; the floriculture company should give appropriate PPE for their workers.

In the present study, floriculture workers had significantly higher levels of (LFTs): ALT, AST, TBL, DBL, and globulin levels, and significantly lower albumin and A/G ratio compared to controls, while ALP and total protein showed no significant difference. These results are generally consistent with evidence from various occupational pesticide exposure studies, though some parameters differed across regions, pesticide types, and study populations. The significant elevation of ALT (27.29 ± 3.30 U/L vs. 22.91 ± 1.72 U/L; P. value < 0.001) and AST (27.99 ± 5.23 vs. 20.37 ± 2.81; P. value < 0.001) in our study is consistent with Thailand (73), Pakistan and Cameron (74), Iraq (75), India (77), Brazil (78), Cameron (80), Benin (81), and Egypt (83).

However, studies in Thailand and Iran showed that ALT and AST did not significantly differ in pesticide-exposed and controls (72, 76). Other studies in Cameron and Tanzania showed that significant increase in ALT but not AST in pesticide-exposed exposed compared to controls (79, 82). The discrepancy may be due to shorter exposure durations, different pesticide formulations, different study setups, and effective use of personal protective equipment.

Our study showed that no significant ALP (78.27 ± 6.51 vs. 78.02 ± 5.59; P- value-0.752) difference between floriculture workers and controls, it lined with studies conducted in Thailand (72) ,and Pakistan and Cameron (74). Our study contrasts with different studies conducted in Thailand (73), Iraq(75), India(77), Cameron(80), and Egypt(83). The variability in ALP findings may be related to pesticide class, with certain compounds more likely to induce cholestatic injury.

In this study, there was a significant increase in TBL (0.87 ± 0.22 vs. 0.37 ± 0.14; P-value <0.001) in pesticide-exposed participants than in controls. Our study aligns with study conducted in Iraq(75). The study contrasts with study conducted in Cameron and Pakistan (74). There was also a significant increase in DBL (0.28 ± 0.10 vs. 0.15 ± 0.06; P-value < 0.001) in floriculture workers than in controls. It was consistent with study conducted in India (77). While it contrasts with studies conducted on Pakistan and Cameroon, and Brazil, with no significant difference between cases and controls (74, 78). Our study suggested that impaired bilirubin metabolism or excretion, potentially due to hepatocellular or canalicular dysfunction. The discrepancy may be due to differences in study setup, pesticide type, and intensity of exposure.

Our study revealed a significant reduction of albumin (4.37 ± 0.40 vs. 4.64 ± 0.26; P-value <0.001) in pesticide-exposed compared to controls, in line with study conducted in Thailand (72). In contrast to our study, the study from India (77) reported increased albumin in exposed workers, potentially reflecting differences in nutritional status, hydration. And also a significant decrease in A/G ratio (1.44 ± 0.34 vs. 1.60 ± 0.26; P-value- 0.001) in pesticide-exposed than controls in our study, which was parallel with (143). Our study indicates that reduced hepatic synthetic capacity under chronic pesticide exposure. Our studies also showed a significant increase of Globulin (3.14 ± 0.46 vs. 2.96 ± 0.41; p. value- 0.019) in pesticide-exposed participants, it line with study conducted in India (77), where chronic inflammatory and immune responses may be proposed as underlying mechanisms.

Our study showed that significantly altered RFTs, where increased urea and creatinine, and BUN to creatinine among pesticide-exposed horticultural workers compared to controls. While eGFR was significantly reduced in pesticide-exposed individuals compared to controls. There was no significant difference in uric acid between pesticide-exposed and controls. These findings suggested potential renal impairment associated with occupational pesticide exposure. In our study, there was a significant increase in urea (40.35 ± 6.40 vs. 20.73 ± 1.80; P-value -<0.001) in pesticide-exposed compared to controls. The study agreed with studies conducted in Iraq (75), India(77), Brazil (78), Cameroon (80, 86), and Egypt (83). While other studies contrast with our studies were study conducted in Thailand (72), Iran (76), Cameron (79), Benin (81), no significant difference between pesticide-exposed and controls. In this study, there was also a significant increase in creatinine (0.92 ± 0.12 vs. 0.59 ± 0.08; P-value <0.001) in floriculture

workers compared to controls, which aligned with different studies conducted in Pakistan and Cameron (74), Brazil (78), Tanzania (82), Cameron (86), and Egypt (83). In contrast, there was no significant difference between pesticide-exposed and controls in other studies conducted in Thailand (72), Iraq (75), Iran (76), India (77), Benin (81), and Cameron (79). These discrepancies may reflect differences in pesticide types, exposure levels, duration, or population susceptibility.

Our study revealed an increased BUN /creatinine ratio (20.68 ± 3.00 vs. 16.86 ± 3.04; P-value <0.001) in floriculture workers than in controls. It might indicate early pre-renal changes, possibly due to dehydration from heat stress combined with pesticide toxicity (144). Other studies conducted in Thailand, and India indicated that there was no significant difference between pesticide-exposed and controls (72, 77). It may be due to differences in pesticide type, and also, they were open-field agricultural studies. Although uric acid levels were slightly higher in cases (4.53 ± 0.76 vs. 4.45 ± 0.76, the difference was not statistically significant (p = 0.478), in line with study conducted in Thailand (72). Other study conducted in India (77) indicated a significant increase in uric acid in cases. It may be due to a difference in pesticide type and duration of exposure. Our study also showed that significant decrease of eGFR (98.84 ± 18.03 vs.132.54 ± 6.86; P-value < 0.001) in cases than controls, in contrast with the study conducted in Thailand(73).

In this study, significantly lower BChE was observed in cases (5285.22 ±1013.29 vs. 6579.00 ±1159.73; P-value <0.001) compared with controls. This was attributed to the presence of OP and CM pesticides, which are commonly used on flower farms in Ethiopia (139, 145, 146). Our study agreed with different studies in Thailand (72), Iraq (75), Brazil (78), and Cameroon (80).

In our study, 9 (17.6 %) of floriculture workers had abnormally low BChE levels. This proportion is consistent with findings from occupational pesticide-exposed other agricultural setting study conducted in Iran which was 23% (76). Our study was lower than the study conducted in Thailand with abnormal Low BChE (46.5%) (84).

In our investigation, floriculture workers had abnormal LFT parameters, including high levels of ALT (11.8%), AST (9.8%), TBL (37.3%), DBL (58.8%), and globulin (23.5%). Conversely,

abnormal reductions were observed in total protein (2%), albumin (2%), and the albumin-to-globulin ratio (3.9%). Other study conducted in India also reported abnormally high values of ALT (16%), AST(12%), ALP (19%), Globulin (6%), and Albumin (4.5%) (77).

In our study, floriculture workers exhibited abnormal RFT parameters, including elevated levels of urea (9.8%), creatinine (29.8%), uric acid (3.9 %), and BUN to creatinine ratio (60.8%). In contrast, a reduced eGFR (<90 Ml/min.1.73 m^2) was observed in 37.3 % of participants, corresponding to stage 2 eGFR impairment. In lined with our study conducted in India (77) showed abnormal high creatinine(4%), urea (15%) , and BUN to creatinine ratio (7%) in pesticide exposed participants. The other study conducted in Thailand (84) indicated that abnormal high creatinine (27.58%), and decreased EGFR <90 Ml/min.1.73 m^2 (24.14%) in pesticide exposed participants.

In our investigation, LFTs, including ALT, AST, TBL, DBL, and globulin levels, increased significantly with longer work duration. Conversely, albumin and the A/G ratio significantly decreased with longer work duration. Workers with > 10 years of exposure had significantly higher ALT, TBL, and DBL levels compared to both the less than 5 years and 5–10 years exposure groups ($p<0.05$). In contrast, albumin and the A/G ratio showed a significant decrease in workers with > 10 years of exposure compared to the other two groups ($p<0.05$). These findings indicate progressive hepatocellular injury associated with chronic pesticide exposure. Prolonged exposure to organophosphate and carbamates pesticides can induce oxidative stress, mitochondrial damage, and disruption of hepatocellular membranes, leading to the leakage of transaminases into the bloodstream (147).

Our study found that RFTs, including urea and creatinine levels, increased significantly with longer work duration, while eGFR showed a significant decline. Workers with more than 10 years of pesticide exposure had significantly higher creatinine levels compared to both the <5 years and 5–10 years exposure groups ($p< 0.05$). Workers with more than 10 years of exposure had significantly higher urea than 5-10 years ($p<0.05$). Conversely, eGFR was significantly lower in workers with >10 years of exposure compared to the <5 years ($p <0.05$). Prolonged pesticide exposure may lead to these changes by directly damaging the distal convoluted tubules,

increasing oxidative stress, inducing rhabdomyolysis, and causing hypovolemia from dehydration (148)

In our study, we assessed the correlation between LFTs, RFTs, and BChE with common associated factors, including age, sex, BMI, PPE use, and work duration among pesticide-exposed participants. In LFTs, age showed a significant positive correlation with ALT, AST, ALP, TBL, and DBL, while it was negatively correlated with albumin and the albumin-to-globulin ratio. Similarly, a study conducted in Egypt reported that age had a strong positive correlation with TBL (97). Our findings indicate that hepatic function declines with increasing age, likely due to cumulative oxidative stress over time. Female sex showed a significant negative correlation with AST, which may reflect sex-related differences in pesticide metabolism and exposure effects. BMI demonstrated significant positive correlations with ALT, DBL and TBL, suggesting a possible contribution of metabolic stress to liver dysfunction. Work duration was positively correlated with ALT, TBL, DBL, and globulin, while negatively correlated with albumin and the A/G ratio. This pattern supports the notion that longer exposure leads to cumulative liver injury and inflammation (147).

In RFTs, age was positively correlated with urea, creatinine, and uric acid, but negatively correlated with eGFR, indicating a progressive decline in renal function with increasing age. Female participants also had significantly lower eGFR values compared to males. In addition, work duration was positively correlated with creatinine, consistent with findings from a study conducted in the Gaza Strip, further supporting the cumulative nephrotoxic effects of prolonged pesticide exposure (85), but significant negative correlation with eGFR, indicating cumulative nephrotoxic effects of pesticides increased with work duration (148).

In BChE, female sex also had a significant negative correlation with BChE, possibly reflecting physiological differences in enzyme activity or susceptibility to pesticide toxicity. Similarly, BMI demonstrated a negative correlation, which may be linked to metabolic alterations influencing pesticide metabolism and enzyme regulation. In contrast, PPE use was positively correlated with BChE, suggesting that consistent use of protective equipment can reduce pesticide-induced BChE inhibition. This finding is supported by evidence from a study

conducted in Brazil, which highlighted the protective role of PPE against cholinesterase inhibition among agricultural workers (149).

In our study, stepwise multiple linear regressions demonstrated that several socio-demographic and occupational factors were significantly associated with abnormalities in LFTs, RFTs, and BChE. Increased work duration was significantly associated with elevated ALT (B = 0.524, $p < 0.001$), AST (B = 0.433, p = 0.039), TBL (B = 0.037, $p < 0.001$), and globulin (B = 0.057, p = 0.005). In contrast, increase work duration was significantly associated with a decrease A/G ratio (B = –0.040, p = 0.007). Increased age was significantly associated with elevated ALP (B = 0.725, p = 0.029), DBL (B = 0.014, p = 0.005). In contrast, increase age was negatively associated with albumin (B- 0.053, p= 0.008). The liver is essential for maintaining the body's homeostasis, performing key functions such as metabolism and detoxification of drugs and xenobiotics (150). The long-term exposure to pesticides is known to exert adverse effects on albumin, ALT, AST, TBL, DBL, globulin resulting in hepatic toxicity (151). Similarly, BMI showed a significant association with increased DBL (B = 0.026, p = 0.004). In addition, female sex was significantly associated with decreased AST levels (B = –5.000, $p < 0.001$), possibly reflecting sex-related physiological differences in liver enzyme activity.

9. Strengths and limitations of the study

9.1. Strength of the study

One of the strengths of this study was that the participants were selected independently, without interference from farm administrators, which enhanced the validity of the findings. The other Strength of the study was that all laboratory analyses were performed at the EPHI Clinical Chemistry Reference Laboratory, the national reference facility, ensuring the reliability and validity of results. Moreover, this is the first biochemical study conducted in Ethiopia, and to our knowledge, the first in East Africa, focusing on workers in the floriculture industry. Additionally, the use of a comparative cross-sectional design, coupled with extensive statistical analyses, including Independent t-test, Kruskual Wallis test, Chi-square, correlation analysis, and stepwise linear regression, further strengthened the validity and robustness of the study outcomes.

9.2. Limitations of the study

There are some limitations to this study. The study cannot demonstrate a clear cause-and-effect relationship between pesticide exposure and the health effects, since research employed a cross-sectional methodology. Recall bias may have been generated since some of the data like reported symptoms and pesticide use habits were dependent on participants' memories. Additionally, because the workers had already been exposed to pesticides regularly, we were unable to get baseline biochemical values before their employment. Smaller sample size due to scarcity of resources. Furthermore, we did not gather comprehensive information on the precise quantities of pesticides applied, the frequency of spraying, or the time after which workers returned to the fields. Finally, the lack of similar studies in Ethiopia and East Africa made it difficult to compare our results with those from other populations

10. Conclusions and recommendations

10.1. Conclusions

The findings of the study showed that different health symptoms, including sweating, skin rash, cough, eye irritations, headache ache and others were reported. Different health problems were also reported, including GIT problems, neurological problems, respiratory problems and skin problems, and others. There was also extensive use of pesticides and fertilizers in our study. More than 34 pesticides were identified, including highly hazardous and slightly hazardous pesticides. There was low supply of PPE and low level of periodical check up in the study area.

The study also revealed that significant increases in levels of LFTs, including ALT, AST, DBL, and globulin compared to controls in floriculture workers. Conversely, significantly low levels of Albumin and A/G ratio in floriculture compared to controls. It indicated that hepatotoxicity of pesticides in floriculture workers. There was also a significant increase in RFTs, including urea, creatinine, and BUN to creatinine in floriculture compared to controls. In contrast, eGFR was significantly decreased in pesticide-exposed participants compared to controls. It showed that nephrotoxicity of pesticides in floriculture workers. Moreover, there was a significant decrease

in BChE level among pesticide-exposed participants compared to controls. It indicated that presence of cholinesterase inhibiting chemicals in our study area.

10.2. Recommendations

- Floriculture industry owners are recommending providing protective full PPE, including gloves, hats, masks, shoes, and a gown to floriculture workers.
- Floriculture industry owners are recommending providing periodical check-ups to floriculture workers for early recognition of health problems.
- Floriculture industry owners are recommending providing training on pesticide usage and the adverse effects of pesticides to the floriculture workers.
- The responsible health sectors should monitor the health status of floriculture workers; provide training and awareness of the toxicity of pesticides.
- Floriculture industry workers are advised to take protective measures at work workplace, including wearing protective equipment.
- Further research is recommended in a longitudinal study with multiple sites of floriculture sites to build up a comprehensive picture of occupational pesticide exposure in Ethiopia.
- Conducting different studies is recommended to include the effect of pesticide-exposed workers on lipid profiles, hormones, electrolytes, AChE, inflammatory markers, and antioxidant enzymes to build a comprehensive effect of occupational exposure in Ethiopia.
- Conducting different studies across other pesticide-exposed populations are recommended as agricultural workers to broaden the effect of pesticides on human health

References

1. Malik M, Bashir E. Horticulture: National book foundation. Islamabad, Pakistan. 1994;633.
2. Edmond JB, Senn TL, Andrews FS, Halfacre R. Fundamentals of horticulture: McGraw-Hill, Inc.; 1975.
3. Shry C, Reiley HE. Introductory horticulture: Cengage Learning; 2016.
4. Mebrat S, Degwale A, Mekonen T, Mebrat A. Flower production prospects and sustainability challenges in Ethiopia: A systematic review. Frontiers in Environmental Science. 2022;10:1026544.
5. Agmas B, Adugna M. Attitudes and practices of farmers with regard to pesticide use in NorthWest Ethiopia. Cogent Environmental Science. 2020;6(1):1791462.
6. Abdou HM, Hussien HM, Yousef MI. Deleterious effects of cypermethrin on rat liver and kidney: protective role of sesame oil. Journal of environmental science and health, part B. 2012;47(4):306-14.
7. Bishop ML. Clinical Chemistry: Principles, Techniques, and Correlations, Enhanced Edition: Principles, Techniques, and Correlations: Jones & Bartlett Learning; 2020.
8. Hall P, Cash J. What is the real function of the liver 'function'tests? The Ulster medical journal. 2012;81(1):30.
9. Hernández AF, Gil F, Tsatsakis AM. Biomarkers of chemical mixture toxicity. Biomarkers in toxicology: Elsevier; 2019. p. 569-85.
10. Guengerich FP. Cytochrome p450 and chemical toxicology. Chemical research in toxicology. 2008;21(1):70-83.
11. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology & therapeutics. 2013;138(1):103-41.
12. Abass K, Turpeinen M, Rautio A, Hakkola J, Pelkonen O. Metabolism of pesticides by human cytochrome P450 enzymes in vitro–a survey. Insecticides—advances in integrated pest management. 2012:165-94.
13. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nature reviews cancer. 2006;6(12):947-60.

14. Hemalatha T, UmaMaheswari T, Krithiga G, Sankaranarayanan P, Puvanakrishnan R. Enzymes in clinical medicine: an overview. 2013.
15. Association AG. American Gastroenterological Association medical position statement: evaluation of liver chemistry tests. Gastroenterology. 2002;123(4):1364-6.
16. Sipos P. Some effects of lead contamination on liver and gallbladder bile. Acta Biologica Szegediensis. 2003;47(1-4):139-42.
17. Li Z, Jiang L, Zhu Y, Su W, Xu C, Tao T, et al. Assessment of hepatic metabolism-dependent nephrotoxicity on an organs-on-a-chip microdevice. Toxicology in Vitro. 2018;46:1-8.
18. Lee F-Y, Chen W-K, Lin C-L, Lai C-Y, Wu Y-S, Lin I-C, et al. Organophosphate poisoning and subsequent acute kidney injury risk: a nationwide population-based cohort study. Medicine. 2015;94(47).
19. Mourad Abu T. Adverse impact of insecticides on the health of Palestinian farm workers in the Gaza Strip: a hematologic biomarker study. International journal of occupational and environmental health. 2005;11(2):144-9.
20. Hardman J, Limbird L, Gilman A. Anticholinesterase agents. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 2001:161-76.
21. Aldridge W. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E 605) and analogues. Biochemical Journal. 1950;46(4):451.
22. Metcalf RL. Session I—The Inhibition of Esterases by Organophosphorus Compounds and Carbamates: Structure—activity relationships for insecticidal carbamates. Bulletin of the World Health Organization. 1971;44(1-2-3):43.
23. Maggi F, Tang FH, la Cecilia D, McBratney A. PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. Scientific data. 2019;6(1):170.
24. Boedeker W, Watts M, Clausing P, Marquez E. RETRACTED ARTICLE: The global distribution of acute unintentional pesticide poisoning: estimations based on a systematic review. BMC public health. 2020;20(1):1875.
25. Tostado L, Bollmohr S. Pesticide Atlas-Facts and figures about toxic chemicals in agriculture. Heinrich-Böll-Stiftung, Friends of the Earth Europe, Bund für Umwelt und …; 2022.

26. Brice K, Patricia A, Norbert NT, Mpoame M. Environmental and human health assessment in relation to pesticide use by local farmers and the Cameroon Development Corporation (CDC), Fako Division, South-West Cameroon. Eur Sci J. 2017;13:454-73.

27. Dhouib IE-B, Lasram MM, Annabi A, Gharbi N, El-Fazaa S. A comparative study on toxicity induced by carbosulfan and malathion in Wistar rat liver and spleen. Pesticide biochemistry and physiology. 2015;124:21-8.

28. Nahhal YE. Biochemical changes associated with long term exposure to pesticide among farmers in the Gaza Strip. Occupational Diseases and Environmental Medicine. 2016;4(03):72-82.

29. Araoud M, Neffeti F, Douki W, Hfaiedh HB, Akrout M, Hassine M, et al. Adverse effects of pesticides on biochemical and haematological parameters in Tunisian agricultural workers. Journal of exposure science & environmental epidemiology. 2012;22(3):243-7.

30. Jamal F, Haque QS, Singh S, Arshad M. The influence of pesticides on hepatic and renal functions in occupational sprayers of rural Malihabad, Lucknow (India). Toxicology: Open Access. 2015;1(01).

31. Khan SM, Sobti R, Kataria L. Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clinica Chimica Acta. 2005;358(1-2):131-8.

32. Kumar J, Lind L, Salihovic S, van Bavel B, Ingelsson E, Lind PM. Persistent organic pollutants and liver dysfunction biomarkers in a population-based human sample of men and women. Environmental research. 2014;134:251-6.

33. Ghazala, Mahboob S, Ahmad L, Sultana S, AlGhanim K, Al-Misned F, et al. Fish cholinesterases as biomarkers of sublethal effects of organophosphorus and carbamates in tissues of Labeo rohita. Journal of Biochemical and Molecular Toxicology. 2014;28(3):137-42.

34. Soltaninejad K, Abdollahi M. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. Medical science monitor: international medical journal of experimental and clinical research. 2009;15(3):RA75-90.

35. Gupta RC. Toxicology of organophosphate and carbamate compounds: Academic Press; 2011.

36. Karami-Mohajeri S, Abdollahi M. Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review. Human & experimental toxicology. 2011;30(9):1119-40.

37. Kumar SV, Fareedullah M, Sudhakar Y, Venkateswarlu B, Kumar EA. Current review on organophosphorus poisoning. 2010.

38. Hoekstra LT, de Graaf W, Nibourg GA, Heger M, Bennink RJ, Stieger B, et al. Physiological and biochemical basis of clinical liver function tests: a review. Annals of surgery. 2013;257(1):27-36.

39. Astiz M, De Alaniz MJ, Marra CA. Effect of pesticides on cell survival in liver and brain rat tissues. Ecotoxicology and environmental safety. 2009;72(7):2025-32.

40. Satar S, Satar D, Tap O, Koseoglu Z, Kaya M. Ultrastructural changes in rat liver treated with pralidoxime following acute organophosphate poisoning. Mount Sinai Journal of Medicine. 2004;71(6).

41. Del Prado-Lu JL. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. Journal of Occupational Medicine and Toxicology. 2007;2(1):9.

42. Mengistie BT. Policy-practice nexus: pesticide registration, distribution and use in Ethiopia. SM J Environ Toxicol. 2016;2(1):1006-19.

43. Breilh J, Pagliccia N, Yassi A. Chronic pesticide poisoning from persistent low-dose exposures in Ecuadorean floriculture workers: toward validating a low-cost test battery. International journal of occupational and environmental health. 2012;18(1):7-21.

44. Silvério ACP, Machado SC, Azevedo L, Nogueira DA, de Castro Graciano MM, Simões JS, et al. Assessment of exposure to pesticides in rural workers in southern of Minas Gerais, Brazil. Environmental Toxicology and Pharmacology. 2017;55:99-106.

45. Abula T, Wondmikun Y. The pattern of acute poisoning in a teaching hospital, north-west Ethiopia. Ethiopian Medical Journal. 2006;44(2):183-9.

46. Abebe M. Organophosphate pesticide poisoning in 50 Ethiopian patients. Ethiopian medical journal. 1991;29(3):109-18.

47. Nigatu AW, Bråtveit M, Moen BE. Self-reported acute pesticide intoxications in Ethiopia. BMC public health. 2016;16:1-8.

48. Organization WH. Endrin: World Health Organization; 1992.

49. Organization WH. Methyl parathion: World Health Organization; 1993.

50. Patil JA, Patil AJ, Govindwar SP. Biochemical effects of various pesticides on sprayers of grape gardens. Indian journal of clinical biochemistry. 2003;18:16-22.

51. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, et al. Worldwide pesticide usage and its impacts on ecosystem. SN Applied Sciences. 2019;1(11):1446.
52. Coman G, Draghici C. Pollutants and their impact on human body. Transilvania University Press, Brasov; 2004.
53. Kassa MA. Review on environmental effects of Ethiopian floriculture industry. Asian Research Journal of Agriculture. 2017;4(2):1-13.
54. Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary toxicology. 2016;9(3-4):90.
55. Tahara M, Kubota R, Nakazawa H, Tokunaga H, Nishimura T. Use of cholinesterase activity as an indicator for the effects of combinations of organophosphorus pesticides in water from environmental sources. Water research. 2005;39(20):5112-8.
56. Badea M, Florescu M, Coman G, Chesca A, Marty J. Comparative studies for pollutants detection using electrochemical sensors and enzyme-based biosensors. Sensing in electroanalysis University Press, Pardubice. 2009;116.
57. Singh S, Kumar V, Thakur S, Banerjee BD, Chandna S, Rautela RS, et al. DNA damage and cholinesterase activity in occupational workers exposed to pesticides. Environmental toxicology and pharmacology. 2011;31(2):278-85.
58. Coman G, Farcas A, Matei AV, Florian C. Pesticides mechanisms of action in living organisms. Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe: Springer; 2013. p. 173-84.
59. Sanchez-Sanchez L, Roman R, Vazquez-Duhalt R. Pesticide transformation by a variant of CYPBM3 with improved peroxygenase activity. Pesticide Biochemistry and Physiology. 2012;102(2):169-74.
60. Costa LG. Current issues in organophosphate toxicology. Clinica chimica acta. 2006;366(1-2):1-13.
61. Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. International journal of environmental research and public health. 2011;8(5):1402-19.
62. Organization WH. The WHO recommended classification of pesticides by hazard and guidelines to classification 2019. The WHO recommended classification of pesticides by hazard and guidelines to classification 20192020.

63. Norkaew S, Siriwong W, Siripattanakul S, Robson M. Knowledge, attitude, and practice (KAP) of using personal protective equipment (PPE) for chilli-growing farmers in Huarua Sub-District, Mueang District, Ubonrachathani Province, Thailand. Journal of health research. 2010;24(suppl 2):93-100.

64. Jambari NSA, Samad NIA, Anua SM, Ruslan R, Hamzah NA. Knowledge, Attitude and Practice (KAP) on pesticide exposure among farmers in Kota Bharu, Kelantan. Malaysian Journal of Medicine & Health Sciences. 2020;16.

65. Jallow MF, Awadh DG, Albaho MS, Devi VY, Thomas BM. Pesticide knowledge and safety practices among farm workers in Kuwait: results of a survey. International journal of environmental research and public health. 2017;14(4):340.

66. Monger A, Mahat K, Dorjee, Om N, Mongar P, Dorji T, et al. Assessment of exposure to pesticides and the knowledge, attitude and practice among farmers of western Bhutan. Plos one. 2023;18(5):e0286348.

67. Yuantari MG, Van Gestel CA, Van Straalen NM, Widianarko B, Sunoko HR, Shobib MN. Knowledge, attitude, and practice of Indonesian farmers regarding the use of personal protective equipment against pesticide exposure. Environmental monitoring and assessment. 2015;187(3):142.

68. Ligani S. Assessments of pesticide use and practice in Bule Hora districts of Ethiopia. Saudi J Life Sci. 2016;1:103.

69. Mequanint C, Getachew B, Mindaye Y, Amare DE, Guadu T, Dagne H. Practice towards pesticide handling, storage and its associated factors among farmers working in irrigations in Gondar town, Ethiopia, 2019. BMC research notes. 2019;12(1):709.

70. Endalew M, Gebrehiwot M, Dessie A. Pesticide use knowledge, attitude, practices and practices associated factors among floriculture workers in Bahirdar City, North West, Ethiopia, 2020. Environmental Health Insights. 2022;16:11786302221076250.

71. Geleta DH, Alemayehu M, Asrade G, Mekonnen TH. Low levels of knowledge and practice of occupational hazards among flower farm workers in southwest Shewa zone, Ethiopia: a cross-sectional analysis. BMC public health. 2021;21(1):232.

72. Aroonvilairat S, Kespichayawattana W, Sornprachum T, Chaisuriya P, Siwadune T, Ratanabanangkoon K. Effect of pesticide exposure on immunological, hematological and

biochemical parameters in Thai orchid farmers—a cross-sectional study. International journal of environmental research and public health. 2015;12(6):5846-61.

73. Bunsri S, Muenchamnan N, Naksen W, Ong-Artborirak P. The hematological and biochemical effects from pesticide exposure on Thai vegetable farmers. Toxics. 2023;11(8):707.

74. Leonel Javeres MN, Habib R, Judith Laure N, Abbas Shah ST, Valis M, Kuca K, et al. Chronic exposure to organophosphates pesticides and risk of metabolic disorder in cohort from Pakistan and Cameroon. International journal of environmental research and public health. 2021;18(5):2310.

75. Othman BA, Kakey ES. environmental pesticide residues and health biomarkers among farmers from greenhouses of Erbil Cucumber crops. Iraqi Journal of Agricultural Sciences. 2020;51(5).

76. Ahmadi N, Mandegary A, Jamshidzadeh A, Mohammadi-Sardoo M, Mohammadi-Sardo M, Salari E, et al. Hematological abnormality, oxidative stress, and genotoxicity induction in the greenhouse pesticide sprayers; investigating the role of NQO1 gene polymorphism. Toxics. 2018;6(1):13.

77. Kori RK, Hasan W, Jain AK, Yadav RS. Cholinesterase inhibition and its association with hematological, biochemical and oxidative stress markers in chronic pesticide exposed agriculture workers. Journal of biochemical and molecular toxicology. 2019;33(9):e22367.

78. Cestonaro LV, Garcia SC, Nascimento S, Gauer B, Sauer E, Göethel G, et al. Biochemical, hematological and immunological parameters and relationship with occupational exposure to pesticides and metals. Environmental Science and Pollution Research. 2020;27(23):29291-302.

79. Manfo FPT, Mboe SA, Nantia EA, Ngoula F, Telefo PB, Moundipa PF, et al. Evaluation of the effects of agro pesticides use on liver and kidney function in farmers from Buea, Cameroon. Journal of toxicology. 2020;2020(1):2305764.

80. Nanhah VJK, Ntepe LJM, Tchuente BRT, Hagbe PV, Goda D, Ekedjoum YDE, et al. Neurological and organ health risks associated with pesticide mixture exposure in banana farm workers in Moungo Division, Cameroon. Scientific Reports. 2025;15(1):21041.

81. Agnandji P, Ayi-Fanou L, Cachon BF, Adjovi ISM, Ouidoh LOH, Sanni A, et al. Influence of pesticide on biochemical and hematological parameters in Beninese vegetable farmers. J Biol Life Sci. 2018;9(1):65-77.

82. Manyilizu WB, Mdegela RH, Kazwala R, Nonga H, Muller M, Lie E, et al. Association of long-term pesticide exposure and biologic parameters in female farm workers in Tanzania: A cross sectional study. Toxics. 2016;4(4):25.

83. Mahmoud Abdul_Aal AA, Mahmoud KW. Assessment of hematological, biochemical and Oxidative Stress parameters on Agricultural Pesticides Application Workers in Egypt. Arab Universities Journal of Agricultural Sciences. 2019;27(2):1619-25.

84. Sombatsawat E, Barr DB, Panuwet P, Robson MG, Siriwong W. Pesticide-induced changes in cholinesterase activity and chronic kidney disease of unknown etiology among farmers in Nakhon Ratchasima, Thailand. Human and Ecological Risk Assessment: An International Journal. 2021;27(8):2038-50.

85. Yassin MM, Al-Shanti TA. Effect of pesticides on kidney function and serum protein profile of farm workers in Gaza Strip. Ann Med Biomed Sci. 2016;2(1):21-7.

86. Tanga M, Telefo P, Tarla D. Alterations in renal functions of market gardeners occupationally exposed to pesticides in West Cameroon. International Journal of Tropical Disease & Health. 2016;20(4):1-10.

87. Allderdice P, Gardner H, Galutira D, Lockridge O, Ladu BN, McAlpine P. The cloned butyrylcholinesterase (BCHE) gene maps to a single chromosome site, 3q26. Genomics. 1991;11(2):452-4.

88. Quin D. Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transitions states. Chem Rev. 1987;78:955-79.

89. Raveh L, Grauer E, Grunwald J, Cohen E, Ashani Y. The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase. Toxicology and applied pharmacology. 1997;145(1):43-53.

90. Simoniello MF, Kleinsorge EC, Scagnetti JA, Mastandrea C, Grigolato RA, Paonessa AM, et al. Biomarkers of cellular reaction to pesticide exposure in a rural population. Biomarkers. 2010;15(1):52-60.

91. Stefanidou M, Athanaselis S, Spiliopoulou H. Butyrylcholinesterase: biomarker for exposure to organophosphorus insecticides. Internal medicine journal. 2009;39(1):57-60.

92. Araoud M, Neffeti F, Douki W, Hfaiedh HB, Akrout M, Najjar MF, et al., editors. Factors influencing plasma butyrylcholinesterase activity in agricultural workers. Annales de biologie clinique; 2011.

93. He F. Biological monitoring of exposure to pesticides: current issues. Toxicology letters. 1999;108(2-3):277-83.

94. Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. Environmental health perspectives. 2004;112(9):950-8.

95. Ntow WJ, Tagoe LM, Drechsel P, Kelderman P, Nyarko E, Gijzen HJ. Occupational exposure to pesticides: blood cholinesterase activity in a farming community in Ghana. Archives of environmental contamination and toxicology. 2009;56(3):623-30.

96. Wafa T, Nadia K, Amel N, Ikbal C, Insaf T, Asma K, et al. Oxidative stress, hematological and biochemical alterations in farmers exposed to pesticides. Journal of Environmental Science and Health, Part B. 2013;48(12):1058-69.

97. Awad O, El-Fiki S, Abou-Shanab R, Hassanin N, Abd El Rahman R. Influence of exposure to pesticides on liver enzymes and cholinesterase levels in male agriculture workers. Global Nest Journal. 2014;16(5):1006-15.

98. Ethiopia FDRo. Central Statistical Agency population projection of Ethiopia for all regions at Woreda level from 2014–2017. Federal Democratic Republic of Ethiopia Addis Ababa, Ethiopia; 2013.

99. Korn DA. Ethiopia, the United States and the Soviet Union. (No Title). 1986.

100. Lewoyehu M. Analysis Of The Level Of Selected Environmental Pollutants In The Effluent Discharges Of Tana Flora Floriculture Industry. Amhara Region, Ethiopia, Nat Prod Chem Res. 2021;9.

101. Heide Fz. Feasibility study for a Lake Tana biosphere reserve, Ethiopia: Deutschland/Bundesamt für Naturschutz; 2012.

102. Bayih MT, Arega Z, Motbainor A. Nutritional status of 6–59 months of age children is not significantly varied between households with and without home gardening practices in Zege, North West Ethiopia, 2020: community based comparative study. BMC pediatrics. 2022;22(1):221.

103. Nigatu AW. Respiratory Health and Acute pesticide intoxications among workers in the flower farm industry in Ethiopia. 2017.

104. Mekonnen YY. Assessment of the Economic and Working Conditions of the Cut Flower Farm Workers in Ethiopia, a Case of Bahir Dar City. Ritsumeikan Asia Pacific University in

Partial Fulfillment of the Requirements for the Degree of Master of Business Administration. 2017.

105. Bergmeyer H, Horder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6. 1.1). Journal of Clinical Chemistry and Clinical Biochemistry. 1986;24(7):497-508.

106. Schumann G, Klauke R, Canalias F, Bossert-Reuther S, FH Franck P, Gella F-J, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) 1. Clinical chemistry and laboratory medicine. 2011;49(9):1439-46.

107. Weichselbaum CT. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American journal of clinical pathology. 1946;16(3_ts):40-9.

108. AW W. Modification of the Malloy-Evelyn method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest. 1972;29:11-2.

109. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. Journal of biological Chemistry. 1937;119(2):481-90.

110. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta. 1971;31(1):87-96.

111. Sampson EJ, Baird MA, Burtis C, Smith EM, Witte D, Bayse DD. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. Clinical chemistry. 1980;26(7):816-26.

112. Suzuki M, Yoshida M. A new enzymatic serum creatinine measurement based on an endogenous creatine-eliminating system. Clin Chim Acta. 1984;143(2):147-55.

113. Schmidt E. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37°C, II. Cholinesterase. Eur J Clin Chem Clin Biochem. 1992;30:163-70.

114. Delgado C, Baweja M, Crews DC, Eneanya ND, Gadegbeku CA, Inker LA, et al. A unifying approach for GFR estimation: recommendations of the NKF-ASN task force on

reassessing the inclusion of race in diagnosing kidney disease. Journal of the American Society of Nephrology. 2021;32(12):2994-3015.

115. Miller WG, Kaufman HW, Levey AS, Straseski JA, Wilhelms KW, Yu HY, et al. National Kidney Foundation Laboratory Engagement Working Group recommendations for implementing the CKD-EPI 2021 race-free equations for estimated glomerular filtration rate: practical guidance for clinical laboratories. Clinical chemistry. 2022;68(4):511-20.

116. Vaidya SR, Aeddula NR. Chronic kidney disease. StatPearls [Internet]: StatPearls Publishing; 2024.

117. Klein G, Lehmann P, Michel E, Regenauer H. Vergleich der IFCC-Methoden für ALAT, ASAT und GGT bei 37 C mit den eingeführten Standardmethoden bei 25 C und 37 C. Lab Med. 1994;18:403-4.

118. Abicht K, El-Samalouti V, Junge W, Kroll M, Luthe H, Treskes M, et al. Multicenter evaluation of new GGT and ALP reagents with new reference standardization and determination of 37 C reference intervals. Clin Chem Lab Med. 2001;39:S346.

119. Josephson B, Gyllenswärd C. The development of the protein fractions and of cholesterol concentration in the serum of normal infants and children. Scandinavian journal of clinical and laboratory investigation. 1957;9(1):29-38.

120. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). European Journal of Clinical Chemistry and Clinical Biochemistry. 1996;34(6):517-20.

121. Pagana KD, Pagana TJ. Mosby's Manual of Diagnostic and Laboratory Tests-E-Book: Mosby's Manual of Diagnostic and Laboratory Tests-E-Book: Elsevier Health Sciences; 2017.

122. Löhr B, El-Samalouti V, Junge W, Maatouk H, Halabi A, Fahle A, et al. Reference range study for various parameters on Roche clinical chemistry analyzers. Clinical laboratory. 2009;55(11-12):465-71.

123. McPherson RA, Pincus MR, Henry JB. Henry's clinical diagnosis and management by laboratory methods. (No Title). 2011.

124. Tietz NW. Clinical guide to laboratory tests. Clinical guide to laboratory tests1995. p. 1096-.

125. Mazzachi BC, Peake MJ, Ehrhardt V. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. Clinical laboratory. 2000;46(1-2):53-5.
126. Thefeld W, Hoffmeister H, Busch E-W, Koller P, Vollmar J. Normalwerte der Serumharnsäure in Abhängigkeit von Alter und Geschlecht mit einem neuen enzymatischen Harnsäurefarbtest. DMW-Deutsche Medizinische Wochenschrift. 1973;98(08):380-4.
127. Blaauwen Dd, Poppe W, Tritschler W. Cholinesterase (EC 3.1. 1.8) mit Butyrylthiocholin-iodid als Substrat: Referenzwerte in Abhängigkeit von Alter und Geschlecht unter Berücksichtigung hormonaler Einflüsse und Schwangerschaft. 1983.
128. Suganya PR, Samuel TR, Rajagopalan B. A study to evaluate the role of BUN/creatinine ratio as a discriminator factor in azotemia. Int J Pharm Sci Rev Res. 2016;40(1):131-4.
129. Zhang CC, Zhang CW, Xing H, Wang Y, Liang L, Diao YK, et al. Preoperative Inversed Albumin-to-Globulin Ratio Predicts Worse Oncologic Prognosis Following Curative Hepatectomy for Hepatocellular Carcinoma. Cancer Manag Res. 2020;12:9929-39.
130. Organization WH. Obesity: preventing and managing the global epidemic: report of a WHO consultation. 2000.
131. Lu S, Robyak K, Zhu Y. The CKD-EPI 2021 equation and other creatinine-based race-independent eGFR equations in chronic kidney disease diagnosis and staging. The journal of applied laboratory medicine. 2023;8(5):952-61.
132. Defar A, Ali A. Occupational induced health problems in floriculture workers in Sebeta and surrounding areas, West Shewa, Oromia, Ethiopia. Ethiopian Journal of Health Development. 2013;27(1):64-71.
133. Al-Sarar A, Abo Bakr Y, Al-Erimah G, Hussein H, Bayoumi A. Hematological and biochemical alterations in occupationally pesticides-exposed workers of Riyadh municipality, Kingdom of Saudi Arabia. Res J Environ Toxicol. 2009;3(4):179-85.
134. Kachaiyaphum P, Howteerakul N, Sujirarat D, Siri S, Suwannapong N. Serum cholinesterase levels of Thai chilli-farm workers exposed to chemical pesticides: prevalence estimates and associated factors. Journal of occupational health. 2010;52(1):89-98.
135. Damalas CA, Koutroubas SD. Farmers' exposure to pesticides: toxicity types and ways of prevention. MDPI; 2016. p. 1.

136. García-García CR, Parrón T, Requena M, Alarcón R, Tsatsakis AM, Hernández AF. Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. Life sciences. 2016;145:274-83.

137. Jamil K, Shaik AP, Mahboob M, Krishna D. Effect of organophosphorus and organochlorine pesticides (monochrotophos, chlorpyriphos, dimethoate, and endosulfan) on human lymphocytes in-vitro. Drug and chemical toxicology. 2005;27(2):133-44.

138. Mrema EJ, Ngowi AV, Kishinhi SS, Mamuya SH. Pesticide exposure and health problems among female horticulture workers in Tanzania. Environmental health insights. 2017;11:1178630217715237.

139. Hanssen VM, Nigatu AW, Zeleke ZK, Moen BE, Bråtveit M. High prevalence of respiratory and dermal symptoms among Ethiopian flower farm workers. Archives of environmental & occupational health. 2015;70(4):204-13.

140. Goodman LS. Goodman and Gilman's the pharmacological basis of therapeutics: McGraw-Hill New York; 1996.

141. Gesesew HA, Woldemichael K, Massa D, Mwanri L. Farmers knowledge, attitudes, practices and health problems associated with pesticide use in rural irrigation villages, Southwest Ethiopia. PloS one. 2016;11(9):e0162527.

142. Zyoud SeH, Sawalha AF, Sweileh WM, Awang R, Al-Khalil SI, Al-Jabi SW, et al. Knowledge and practices of pesticide use among farm workers in the West Bank, Palestine: safety implications. Environmental health and preventive medicine. 2010;15(4):252-61.

143. Patil JA, Patil AJ, Sonttake A, Govindwar SP. Occupational pesticides exposure of sprayers of grape gardens in western Maharashtra (India): effects on liver and kidney function. Journal of basic and clinical physiology and pharmacology. 2009;20(4):335-56.

144. Bodin T, García-Trabanino R, Weiss I, Jarquín E, Glaser J, Jakobsson K, et al. Intervention to reduce heat stress and improve efficiency among sugarcane workers in El Salvador: Phase 1. Occupational and environmental medicine. 2016;73(6):409-16.

145. Negatu B, Kromhout H, Mekonnen Y, Vermeulen R. Use of chemical pesticides in Ethiopia: a cross-sectional comparative study on knowledge, attitude and practice of farmers and farm workers in three farming systems. Annals of Occupational Hygiene. 2016;60(5):551-66.

146. Shentema MG, Kumie A, Bråtveit M, Deressa W, Ngowi AV, Moen BE. Pesticide use and serum acetylcholinesterase levels among flower farm workers in Ethiopia—a cross-sectional study. International journal of environmental research and public health. 2020;17(3):964.

147. Sule RO, Condon L, Gomes AV. A Common Feature of Pesticides: Oxidative Stress-The Role of Oxidative Stress in Pesticide-Induced Toxicity. Oxid Med Cell Longev. 2022;2022:5563759.

148. Sahu V, Panjwani D, Mishra V. A Review of Nephrotoxins and Their Mechanism of Nephrotoxicity. Current Functional Foods. 2024;2(2):E260124226378.

149. Remor AP, Totti CC, Moreira DA, Dutra GP, Heuser VD, Boeira JM. Occupational exposure of farm workers to pesticides: biochemical parameters and evaluation of genotoxicity. Environment international. 2009;35(2):273-8.

150. Lozano-Paniagua D, Parrón T, Alarcón R, Requena M, López-Guarnido O, Lacasaña M, et al. Evaluation of conventional and non-conventional biomarkers of liver toxicity in greenhouse workers occupationally exposed to pesticides. Food and Chemical Toxicology. 2021;151:112127.

151. Madani FZ, Hafida M, Merzouk SA, Loukidi B, Taouli K, Narce M. Hemostatic, inflammatory, and oxidative markers in pesticide user farmers. Biomarkers. 2016;21(2):138-45.

152. Moss D, Henderson A, Kachmar J. Enzymes in: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia: WB Saunders; 1987.

153. Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. Journal of biological chemistry. 1946;164(1):321-9.

154. GRANT GH. Amino acids and proteins. Fundamentals of clinical chemistry. 1987.

155. Balistreri W. Liver function. Fundamentals of clinical chemistry. 1987:729-61.

156. Lamb EJ, Jones GR. Kidney function tests. Tietz textbook of clinical chemistry and molecular diagnostics 6th ed St Louis (MO): Elsevier Saunders. 2017:479-517.

157. Lamb EJ, Tomson CR, Roderick PJ. Estimating kidney function in adults using formulae. Annals of clinical biochemistry. 2005;42(5):321-45.

158. Kageyama N. A direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. Clin Chim Acta. 1971;31(2):421-6.

159. Town M-H, Gehm S, Hammer B, Ziegenhorn J. A sensitive colorimetric method for the enzymatic determination of uric acid. J Clin. 1985;23:591.

Annexes

Annex I: Information sheet

A. Information sheet in English Version

Title of the Research Project: Assessment of liver and renal function among horticulture workers at Tana Flora Floriculture Industry, Bahirdar, Ethiopia, 2025.

Principal Investigator: Habtamu Molla Gietie (BSc, MSc candidate)

Name of the Organization: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science.

Introduction

Hello! My name is Habtamu Molla, and I am an MSc student at Addis Abeba University College of Health Science, Department of Medical Laboratory Technology. I am doing my final research for graduation on the assessment of Liver and Renal function among horticulture workers at Tana Flora Floriculture Industry, Bahirdar, Ethiopia. You are invited to participate as a study subject in research conducted by an MSc candidate from Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator, four advisors, all from Addis Ababa University's clinical chemistry department. Please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because the aim of this study is to assess liver, renal function among horticulture workers at Tana Flora Floriculture Industry, Bahirdar, Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this, but also specimens collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staff as it is needed. 5 microliters of blood will be collected by the principal investigator or other trained data collector. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from a venous puncture. Moreover, there will be a face-to-face interview for additional questions.

Potential Risks and Discomforts

During the collection of specimens from you, appropriate precautions will be taken, and all samples will be collected by trained health professionals. If anything happened, appropriate medical care would be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet or will be protected by a password on the computer, only accessible to personnel involved in the study. There is no sensitive issue that you will be asked about related to your social desirability, but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result, you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and management of exposure to pesticides. Hence, you are indirectly benefiting other patients and society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary, and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also refuse to give

any sample. You can ask any questions regarding this study, and you have the right to get a laboratory diagnosis result for free.

Contact information

If you have any questions about this study, you can contact the following principal investigators and advisors for further information.

Habtamu Molla Phone: 0964689051

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B. የ ተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

የምርምር ፕሮጀክቱ ርዕስ፡ Assessment of liver and renal function among horticulture worker at Tana flora floriculture industry, Bahirdar, Ethiopia.

ዋና ተመራማሪ፡ ሀብታሙ ሞላ (የመጀመሪያ ድግሪ፣ እጩ ማስተር)

የድርጅቱ ስም፡- አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል።

መግቢያ

ሀሎ! ሀብታሙ ሞላ እባላለሁ በአዲስ አበባ ዩኒቨርሲቲ የሜዲካል ላብራቶሪ ቴክኖሎጂ የጤና ሳይንስ ክፍል ኮሌጅየMSc ተማሪ ነኝ። በ" Assessment of liver and renal function among horticulture worker at Tana flora floriculture industry, Bahirdar, Ethiopia "ለመመረቅ የመጨረሻ ምርምሬን እያደረግሁ ነው። ከአዲስ አበባ ዩኒቨርሲቲ በ ማስተር እጩ በሚደረገው ጥናት እንድትሳተፉ ተጋብዘዋል። የእርስዎ ተሳትፎ በፈቃደኝነት ነው።የምርምር ቡድኖቹ አንድ ዋና መርማሪ, አራት አማካሪዎች; አራቱምቱ ከአዲስ አበባ ዩኒቨርሲቲ ክሊኒካል ኬሚስትሪ ክፍል ይሆናሉ። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡

የምርምር ፕሮጀክቱ ዓላማ

በዚህ ጥናት ላይ እንድትሳተፉ እንጠይቃችኋለን ምክንያቱም የዚህ ጥናት አላማ ጉበት እና የኩላሊት ተግባርን በሆርቲካልቸር ሰራተኞች መካከል ያለውን የጣና ፍሎራ አበባ ኢንዱስትሪ ባህርዳር፣ ኢትዮጵያ፣ ለመገምገም ነው።

ሂደቶች እና የሚጠበቀው ተሳትፎ

ለመሳተፍ ፍቃደኛ ከሆንክ/ሽ የጥናቱ አላማ ተረድተህ ፍቃድህን መስጠት አለብህ። ይህ ብቻ ሳይሆን ከእርስዎ የሚሰበሰቡ ናሙናዎችም ለምርምር ዓላማ የሚውሉ ሲሆን የናሙናዎ ውጤትም እንደ አስፈላጊነቱ ለሚመለከታቸው ባለሙያዎች ይጋለጣል። 5 ማይክሮ ሊትር ደም የሚሰበሰበው በዋናው መርማሪ ወይም ሌላ የሰለጠነ መረጃ ሰብሳቢ ነው። ከዚያ፣ ፈቃድዎን እንዲሰጡ ተጠይቀዋል። ናሙና ሰብሳቢው ከስምምነት በኋላ ናሙና ከ venious puncture የደም ናሙና ይወሰዳል። ከዚህም በላይ ለተጨማሪ ጥያቄዎች ፊት ለፊት ቃለ መጠይቅ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ከእርስዎ ናሙና በሚሰበሰብበት ጊዜ ተገቢውን ጥንቃቄ ይደረጋል እና ሁሉም ናሙናዎች በሰለጠኑ የጤና ባለሙያዎች ይሰበሰባሉ። ፡ ነገር ግን ደም በሚሰጡት ሰኣት በጣም ትንሽዬ ህመም ሊሰማዎት ይችላል። የሆነ ነገር ከተፈጠረ, ተገቢ የሕክምና እንክብካቤ ይሰጥዎታል።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

የእርስዎን ግላዊነት እና ሚስጥራዊነት እናከብራለን። የ እርስዎን ማንኛውም መረጃ ከአጥኚ ቡድኑ ውጭ ለሌላ ለማንም አይጋራም። እንደ ጥናቱ አካል ከእርስዎ የምንሰበስበው መረጃ በተቆለፈ የፋይል ቁም ሣጥን ውስጥ ይቀመጣል ወይም በኮምፒዩተር ላይ በጥናቱ ውስጥ ለሚሳተፉ ሠራተኞች ብቻ በሚደረስ የይለፍ ቃል ይጠበቃል። ከማህበራዊ ፍላጎትዎ ጋር የተያያዘ ምንም አይነት ጥንቃቄ የሚጠይቅ ጉዳይ ሁሉ በ ሚስጥር ይያዛል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

በዚህ የጥናት ጥናት እንደ ማካካሻ ለመሳተፍዎ ምንም አይነት ክፍያ አያገኙም። ነገር ግን, በምርመራው ውጤት ላይ በመመርኮዝ ከዚህ አንጻር ህክምና ይደረግልዎታል።በተጨማሪም የጥናቱ ውጤት ለፀረ-ተባይ መድሐኒት ተጋላጭነትን ለመለየት እና ለመቆጣጠር ጠቃሚ ይሆናል.። ስለሆነም በተዘዋዋሪ መንገድ ሌሎች ታካሚዎችንና ህብረተሰቡን በዚህ ረገድ እየጠቀማችሁ ነው።

ተሳትፎ እና ከጥናቱ የመውጣት መብት

ተሳትፎው በፈቃደኝነት ነው እናም በዚህ ጥናት ውስጥ ላለመሳተፍ መብት አለዎት። በማንኛውም ጊዜ እና ቦታ ምንም አይነት መዘዝ ሳይኖር ከ ጥናቱ መዉታት ይችላሉ። እንዲሁም ማንኛውንም ናሙና መስጠት አለመቀበል ይችላሉ። ይህንን ጥናት በተመለከተ ማንኛውንም ጥያቄ መጠየቅ ይችላሉ እና የላብራቶሪ ምርመራ ውጤት በነጻ የማግኘት መብት አለዎት።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ስለዚህ ጥናት ማንኛውም አይነት ጥያቄ ካሎት ለበለጠ መረጃ የሚከተሉትን ዋና መርማሪዎች እና አማካሪዎችን ማነጋገር ይችላሉ።

ሀብታሙ ሞላ ስልክ 0964689051

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ፕሮፌሰር ምስጢረ ወልዴ (ፒኤችዲ፣ ፕሮፌሰር)

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ኢሜል፡ abenegesso@gmail.com

ጎበና ደደፎ (ባችለር፣ማስተር)

ኢሜል፡gobenadedefo@gmail.com

መቅደስ አለም (ባችለር፣ማስተር)

ኢሜል፡ mk.alem12@gmail.com

Annex II: Informed consent form in English version

A. Informed consent form in English version

ID No …..……

I had been informed that the objective of this study is to assess Liver and renal function among horticulture workers at Tana Flora Floriculture Industry. The results of this study have an important role in treating me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing renal function, liver function among pesticide-exposed workers in horticulture in Ethiopia. I had also been informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data, which includes blood and a filling questionnaire. Therefore, with a full understanding of the importance of the study, I agreed voluntarily to provide the requested samples, and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

B. የተሳታፊዎች ስምምነት ማረጋገጫ

መለያ ቁጥር ……

የዚህ ጥናት ዓላማ በጣና ፍሎራ የአበባ ልማት ኢንዱስትሪ ውስጥ በሆርቲካልቸር ሠራተኛ መካከል ያለውን የጉበት፣ የኩላሊት ተግባር መገምገም እንደሆነ ነዉ። የዚህ ጥናት ውጤት እኔን እና ሌሎች ታማሚዎችን ለማከም እና ለወደፊት ስልቶች ወይም መመሪያዎች በኢትዮጵያ ውስጥ በአትክልትና ፍራፍሬ ውስጥ ከሚገኙ ፀረ ተባይ ማጥፊያ ሰራተኞች መካከል የጉበት ተግባር እና የኩላሊት ተግባር ለመመርመር እንደ ግብአት ይጠቅማል። የዚህ ጥናት ምስጢራዊነት ተነግሮኛል። ዋናው መርማሪ የደም እና የመሙያ መጠይቅን ያካተተ አስፈላጊውን መረጃ ለማቅረብ ፈቃደኛ መሆንን በሚጠይቀው ጥናት ላይ እንድሳተፍ ጠየቀኝ። ስለሆነም የጥናቱን አስፈላጊነት ሙሉ በሙሉ በመረዳት የተጠየቁትን ናሙናዎች ለማቅረብ በፈቃደኝነት ተስማምቼያለሁ እና የእኔ ጥቅም ከነጻ የላብራቶሪ ምርመራ ውጤት ብቻ ይሆናል።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

_____ፊርማ፡

_____ ቀን_____

Annex III: Questioner

A. Questioner in the English version

Hi, my name is Habtamu Molla, and I am an MSc Medical Laboratory Sciences student at Addis Ababa University College of Health Sciences. I am doing my final thesis on the Assessment of liver, renal function among horticulture workers at Tana Flora Floriculture Industry, Bahirdar, Ethiopia. Whatever information you provide will be kept strictly confidential. Are you a volunteer?

Yes _____ No _____

Instructions:

- Writing the respondent's name is not needed.
- Fill the blank space with appropriate words
- Circle options if space is not given
- All the information collected will be confidential

Date of interview _____

Code number _____

Note: Please encircle or write the appropriate answer in the space provided.

I. Socio-demographic characteristics

101. Sex: 1. Male 2. Female

102. Age (Years): _____

1. 18-25 2. >25

103. Marital status:

(1) Married (2) Single (3) Widowed (4) Divorced.

104. What is your level of education?

(1) Illiterate (2) reading & writing (3) elementary (4) Secondary School (5) Diploma/University and above\

105. Employment

1. Permanent 2. Temporary

106. Monthly income (Ethiopian birr) ----------

1. <1500 2. 1500- 3000 3, 3000-5000 4, > 5000

107. Residency

1. Urban 2. Rural

II. Health-related associated factors

201. How long have you worked in the horticultural area? ----------

(1) < 5 (2) 5-10 years (3), >10 years

202. How many hours do you work at this hour per day…….

203. Do you have a history of any liver or kidney problems before hiring for this work?

(1) Yes (2) No

204. What types of symptoms did you experience after the post-application of pesticides?

(1) Skin rash (2) Eye irritation (3) burning sensation of face (4) sweating (5) cough (6) Headache (7) Other

205. Do you have foot edema? 1. Yes 2. No

206. If your answer is yes, was the foot edema present before you were employed in this company?

1. Yes 2. No

207. How do you describe your current health situation?

(1), Excellent (2), Very good (3) , Fair (4), Poor (5), Very poor

208. Working environment.

1, Flower cutting 2, packing 3, Spraying 4, Irrigation

209. Body mass index (weight in Kg/height in M^2)_____height--------weight-------

1. <18.5 kg/M^2 2. 18.5-24.9 kg/M^2 3. 25-30 kg/M^2 4. > 30 kg/M^2

210. Health problems you faced related to pesticide exposure.

1, Skin problem 2, Respiratory problem 3, Neurological problem 4, Gastrointestinal problem 5. Reproduction problem 6. Eye problem 7.Other

III. Assessment of knowledge, aptitude and practice to pesticide exposure

A. <u>Knowledge</u>

301. Do you know pesticides by name? 1, yes 2, No

302. Do you read and understand pesticide labels? 1, yes 2, No

303. Do you know pesticide-related health problems? 1, yes 2, No

304. Do you know routes of exposure? 1. Yes 2. No

305. If yes, what are the routes of exposure?

1, skin contact 2, ingestion 3, eye contact 4, inhalation

B. <u>Attitude</u>

306. Do you think all pesticides have adverse effects on human health?

1, Strongly agree 2, Agree 3, Disagree 4, Strongly Disagree

307. Do you think pesticides should be discouraged?

1, Strongly agree 2, Agree 3. Disagree 4, Strongly disagree

308. Do you think personal protective equipment prevents pesticide exposure?

1, Strongly agree 2, Agree 3, Disagree 4, Strongly disagree

309. Do you think wearing Personal protective equipment is important?

1, Strongly agree 2, Agree 3. Disagree 4, Strongly disagree

310. Do you think, good pesticide handling reduces health problems?

1, Strongly agree 2, Agree 3. Disagree 4, Strongly disagree

311. Opinion about the negative impact of pesticides on self's health

(1) Pesticide has no health effect (2) pesticide has a minor effect (3) pesticide has a major effect (4) pesticides are fatal

C. <u>practice</u>

312. Do you use personal protective equipment? 1, yes 2, No

313. If yes, which personal protective equipment have you used at your workplace?

1. Gloves 2. Hat 3. Respirator/Mask 4. Special shoes 5. Gown 6.Eye goggle

314. If you have not worn any of the equipment listed above, what is the reason?

(1) Not provided (2) Not-comfortable (3) Not necessary (4) Carelessness (5) Other, specify

315. Do you take a bath after pesticides are applied? 1, yes 2 , No

316. Do you wash your hands immediately after work? (1), yes (2) no

317. Do you change clothes before going home? 1, yes 2, No

318. Is there safe pesticide storage in working area 1, yes 2, No

319. During work, are you doing the following?

1. Drinking 2. Eating 3. Chewing gum

320. Do you smoke tobacco/cigarettes? (1) Yes (2) No

321. If your answer is Yes, how many cigarettes on average do you smoke per day?--

322. Do you consume coffee? (1) Yes (2) No

323. Do you consume alcohol? (1) yes (2) no

324. What types of methods do you use to eliminate or dispose of empty pesticide containers?

(1) Throw in the field (2) throw in the river (3) bury in the ground (4) burn

325. What action to be taken after pesticide exposure?

1. Go to health facility 2, stop working, and rest 3, Traditional medicine

326. Do you take pre pre-medical checkup? 1. Yes 2. No

327. Do you take training before work (pre-training)? 1, Yes 2, No

328. Do you take a periodic checkup? 1, yes 2, No

329. Do you have a supply of personal protective equipment? 1, yes 2, No

330. If yes, what types of personal protective equipment?

1, Mask 2, Boot 3, Eye google 4, gown 5, cap

331. Do you take on job training? 1, yes 2, No

B. መጠይቅ በአማርኛ ቅጂ

ሰላም ሀብታሙ ሞላ እባላለሁ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የኤምኤስሲ ሜዲካል ላብራቶሪ ሳይንስ ተማሪ ነኝ። በባህር ዳር ኢትዮጵያ በሚገኘዉ ጣና ፍሎራ አበባ ልማት ኢንዱስትሪ ውስጥ በሆርቲካልቸር ሰራተኛ ጉበት፣ የኩላሊት

ተግባር ግምገማ ላይ የመጨረሻ ጥናቴን እየሰራሁ ነው ያቀረቡት ማንኛውም መረጃ በጥብቅ በሚስጥር ይጠበቃል። በጎ ፈቃደኛ ነህ?

አዎ _____ አይ _____

መመሪያዎች፡-

- የምላሽ ስም መጻፍ አያስፈልግም።
- ባዶውን ቦታ በተገቢው ቃላት ሙላ
- ቦታ ካልተሰጠ የክበብ አማራጮች
- ሁሉም የሚሰበሰቡት መረጃዎች ሚስጥራዊ ይሆና

ቃለ መጠይቁ የሚሰበስበዉ ስም_____ፊርማ_____

የቃለ መጠይቁ ቀን _____

ኮድ ቁጥር _____

ማሳሰቢያ፡ እባኮትን ክበቡ ወይም ተገቢውን መልስ በተሰጠው ቦታ ላይ ይፃፉ።

I. ስነ ህይወታዊ ባህርያት

101. ጾታ፡ 1. ወንድ 2. ሴት

102. ዕድሜ (ዓመታት)፡ _____

1. 18-25 ዓመት 2. › 25 ዓመት

103. የጋብቻ ሁኔታ፡.

(1) ያገባ (2) ያላገባ (3) ባል የሞተባት (4) የተፋታ።

104. የትምህርት ደረጃዎ ስንት ነው?

(1) ማንበብ እና መጻፍ የማይችል (2) ማንበብ እና መፃፍ (3) አንደኛ ደረጃ (4) ሁለተኛ ደረጃ ትምህርት ቤት (5) ዲፕሎማ/ዩኒቨርሲቲ እና ከዚያ በላይ

105.የ ሥራ ሁኔታ

1. ቋሚ 2. ጊዜያዊ

106. ወርሃዊ ገቢ (የኢትዮጵያ ብር)

1, <1500 2. 1500- 3000 3, 3000-5000 4, > 50000

II. **ከጤና ጋር የተዛመዱ ምክንያቶች**

201. በሆርቲካልቸር (በ አበባ ልማት ዉስት) ለምን ያህል ጊዜ ሰርተዋል? (የሥራ ዓመታት) _____

(1)፣ <5 ዓመት (2) 5-10 ዓመት (3)፣ > 1 o ዓመት

202. በቀን ስንት ሰአት ይሰራሉ _____

203. ወደዚህ ሥራ ከመቅጠርዎ በፊት የጉበት ወይም የኩላሊት ችግር አጋትምዎት ያቃል?

(1) አዎ (2) አይደለም

204. ፀረ-ተባይ መድሃኒቶችን ከተጠቀሙ በኋላ ምን አይነት ምልክቶች አጋጥምዎታል?

(1) የቆዳ ሽፍታ (2) የዓይን ህመም (3) የፊት መቃጠል ስሜት (4) ላብ (5) ሳል (6) ሌላም ይግለጹ----

205. የእግር እብጠት አለብዎት? 1. አዎ 2. አይደለም

206. መልስዎ አዎ ከሆነ በዚህ ኩባንያ ውስጥ ከመቀጠርዎ በፊት የእግር እብጠት ነበር?

1. አዎ 2. አይደለም

207. አሁን ያለዎትን የጤና ሁኔታ እንዴት ይገልጹታል?

(1) ፣ እጅግ በጣም ጥሩ (2) ፣ በጣም ጥሩ (3) ፣ መካከለኛ (4) ፣ ጥሩ ያልሆነ (5) ፣ በጣም ደካማ

208. በ አበባ ልማቱ ዉስጥ የስራ ቦታዎ

1, አበባ መቁረጥ 2, ማሸግ 3, ርጭት 4, መሰኖ

209. የሰውነት ክብደት መረጃ ጠቋሚ (ክብደቱ በኪ.ግ/ቁመት በ M2) _____ቁመት _____ክብደት _____

1. <18.5 kg/M^2 2. 18.5-24.9 kg/M^2 3. 25-30 kg/M^2 4. > 30 kg/M^2

210. ከፀረ-ተባይ መጋለጥ ጋር በተያያዘ ያጋጠሙዎት የጤና ችግሮች.

1, የቆዳ ችግር 2, የመተንፈስ ችግር 3, የአእምሮ ችግር 4, የጨጓራ እና የአንጀት ችግር 5. የመራቢያ ችግር 6. ሌሎች ይግለጹ ----

III. ለፀረ-ተባይ መጋለጥ የእውቀት, የችሎታ እና ተግባር ግምገማ

ሀ. እውቀት

301. ፀረ ተባይ መድኃኒቶችን በስም ያዉቃሉ? 1፣ አዎ 2፣ አላቅም

302. በ ፀረ ተባይ መድሃኒቶች ላይ ያለዉን መረጃያ አንብበዉ ይረዱታል? 1፣ አዎ 2፣ አላቅም

303. ከተባይ ተባይ ጋር የተያያዙ የጤና ችግሮችን ያውቃሉ? 1፣ አዎ 2፣ አላቅም

304. ለ ጸረ ተባይ የሚያጋልጡ መንገዶችን ያውቃሉ? 1. አዎ 2, አይደለም

305. አዎ ከሆነ፣ የተጋላጭነት መንገዶች ምንድናቸው?

1, የቆዳ ንክኪ 2, ወደ ውስጥ መግባት 3, የዓይን ንክኪ 4, እስትንፋስ

ለ፣ አመለካከት

306. ሁሉም ፀረ-ተባይ መድሃኒቶች በሰው ጤና ላይ አሉታዊ ተጽእኖ ያሳድራሉ ብለው ያስባሉ?

1, በጣም እስማማለሁ 2, እስማማለሁ 3, አልስማማም 4, በጣም አልስማማም

307. ፀረ-ተባይ መድሃኒት መበረታታት የለበትም ብለዉ ይመስልዎታል?

1, በጣም እስማማለሁ 2, እስማማለሁ 3. አልስማማም 4, በጣም አልስማማም

308. የግል ጸረ ተባይ መከላከያ መሳሪያዎች ከፀረ ተባይ መጋለጥ ይከላከላሉ ብለው ያስባሉ?

1, በጣም እስማማለሁ 2, እስማማለሁ 3, አልስማማም 4, በጣም አልስማማም

309. የግልጸረ ተባይ መከላከያ መሳሪያዎችን መልበስ አስፈላጊ ነው ብለው ያስባሉ?

1, በጣም እስማማለሁ 2, እስማማለሁ 3. አልስማማም 4, በጣም አልስማማም

310. ጥሩ ፀረ-ተባይ አያያዝ የጤና ችግሮችን ይቀንሳል ብለው ያስባሉ?

1, በጣም እስማማለሁ 2, እስማማለሁ 3. አልስማማም 4, በጣም አልስማማም

311. ፀረ-ተባይ መድሃኒቶች በራስ ጤና ላይ አሉታዊ ተፅእኖን በተመለከተ አስተያየት

(1) ፀረ ተባይ መድሐኒት የጤና ችግር የለውም (2) ፀረ ተባይ መድሐኒት አነስተኛ ችግር አለው (3) ፀረ ተባይ መድሐኒት ከፍተኛ ጉዳት አለው (4) ፀረ ተባይ መድኃኒቶች ገዳይ ናቸው።

ሐ. ተግባር

312. የግል ጸረ ተባይ መከላከያ መሳሪያዎችን ትጠቀማለህ? 1፣ አዎ 2፣ አልተጠቀመም

313. አዎ ከሆነ፣ በስራ ቦታዎ ላይ የሚከተለውን የግል ጸረ ተባይ መከላከያ ምን ያህል ጊዜ ይለብሳሉ?

1. ጓንቶች 2. ኮፍያ 3. መተንፈሻ/ጭንብል 4. ልዩ ጫማዎች 5. ጋውን

314. ከላይ ከተዘረዘሩት መሳሪያዎች ውስጥ አንዱንም ካልለበሱ ምክንያቱ ምንድን ነው?

1) አቅርቦት ስለሌለ (2) ስለ ማይመች (3) አስፈላጊ አይደለም (4) ግድየለሽነት (5) ሌላ፣ ይግለጹ_____

315. ፀረ ተባይ መድሃኒት ከነኩ በኋላ ሰዉነትዎን ይታጠባሉ? 1፣ አዎ 2፣ አልታጠብም

316. ከስራ በኋላ ወዲያውኑ እጅዎን ይታጠቡ? (1)፣ አዎ (2) አልታጠብም

317. ወደ ቤት ከመሄድዎ በፊት ልብስ ይለውጣሉ? 1፣ አዎ 2፣ አለዉጥም

318. በሥራ ቦታ ደህንነቱ የተጠበቀ ፀረ-ተባይ ማከማቻ አለ? 1፣ አዎ 2፣ የለም

319. በስራ ወቅት የሚከተሉትን ነገሮች እያደረጉ ነው?

1. ይጠጣሉ 2. ይበላሉ 3. ማስቲካ ያኝካሉ

320. ትምባሆ/ሲጋራ ያጨሳሉ ? (1) አዎ (2) አላጨስም

321. መልስዎ አዎ ከሆነ፣ በቀን ስንት ሲጋራ ያጨሳሉ ?_____

322. ቡና ይጠጣሉ? (1) አዎ (2) አልጠጣም

323. አልኮል ይጠከማሉ? (1) አዎ (2) አልጠቀምም

324. ባዶ ፀረ ተባይ መያዣን ለማስወገድ ምን አይነት ዘዴ ይጠቀማሉ?

(1) በሜዳው ውስጥ መጣል (2) በወንዝ ውስጥ መጣል (3) መሬት ውስጥ መቅበር (4) ማቃጠል

325. ለፀረ-ተባይ ከተጋለጡ በኋላ ምን አይነት እርምጃ መውሰድ ይመርጣሉ?

1. ወደ ጤና ተቋም መሄድ 2, ስራ ማቆም እና ማረፍ 3, የባህል ህክምና 4፣ ሌላ_____

326. ከስራ በፊት ቅድመ ሕክምና ምርመራ አድርገዋል ? 1. አዎ 2, አላደረኩም

327. ከስራ በፊት ቅድመ ስልጠና ወስደዋል ? 1፣ አዎ 2፣ አልወሰድኩም

328. በ የተወሰነ ሰአት ምርመራ ያደርጋሉ? 1፣ አዎ 2፣ አላደግም

329. የግል ጸረ ተባይ መከላከያ መሳሪያዎች አቅርቦት አለዎት? 1፣ አዎ 2፣ የለኝም

330. አዎ ከሆነ፣ ምን አይነት የግል ጸረ ተባይ መከላከያ መሳሪያዎች አለወት ?

1፣ ማስክ 2፣ ልዩ ጫማ 3፣ የአይን መነጸር 4፣ ጋውን 5፣ ኮፍያ

331. የስራ ላይ ስልጠና ወስደዋል? 1፣ አዎ 2፣ አልወሰድኩም

ስለተሳተፉ እጅግ አድርገን እናመሰግናለን !!!!

Annex IV: Laboratory procedures

SOP for blood collection

Equipment

- 21-gauge needle for each participant
- Blood collection tubes (serum separator tube)
- Tourniquet
- Box of nitrile/vinyl gloves
- 70% alcohol
- Cotton
- Safety box

Laboratory Blood sample collection procedure and processing

1. Assemble blood collection materials.
2. Identify and prepare the patient.
3. Label tubes with the client's name/identification number.
4. Wear the rubber gloves and place the patient in a comfortable position
5. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
6. Using the tip of the index finger, examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture.

7. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alco alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 3ml into a serum separator tube.
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe container.
11. Leave for 30 to 45 minutes to clot the blood.
12. Centrifuge at 3000 rpm for 5 minutes, and the Serum will separate
13. Then turn on the clinical chemistry analyzer machine
14. Check the expiry date of all reagents
15. Check the daily, weekly, monthly, quarterly, and yearly controls, standards, and
16. Calibration results of the analyzer (CFAS (Calibration for Automated system)
17. Perform Controls (PreciControl ClinChem Multi 1) PCCM1 and (PreciControl ClinChem Multi 2) PCCM2 controls.
18. The PCCM1 (low control) and PCCM2 (high control) were interpreted with the Westerguard rules.
19. After the QC passes the westerguard rule, analyze the specimen based on the leaflet procedure for each parameter.
20. Finally, the result was printed from the auto machine

Annex V: Principles of the laboratory tests

1. Liver function tests

1.1.Alanine aminotransferase (ALT)

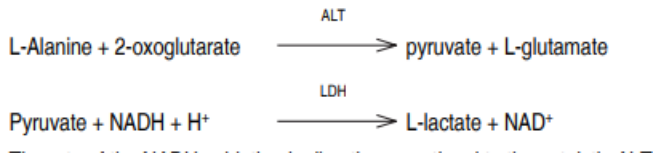
Test Kit name: ALTL

Purpose

Numerous tissues have been found to contain the ALT. Because the liver is the primary source of ALT, ALT activity is measured to diagnose hepatic disorders. Hepatitis, cirrhosis, obstructive jaundice, liver cancer, and long-term alcohol misuse are all associated with elevated serum ALT levels. Patients with a simple myocardial infarction have ALT that is just marginally increased (152).

Principles

The principle of ALT measurement is based on the International Federation of Clinical Chemistry (IFCC) without the pyridoxal phosphate method. Alpha-ketoglutarate and L-alanine react to produce L-glutamate and pyruvate when ALT catalyzes the reaction. Pyruvate changes to lactate and NADH to NAD when LDH is present. The serum activity of ALT is directly proportional to the decrease in NADH absorbance, as measured at 340 nm. The reaction has a kinetic rate (105).



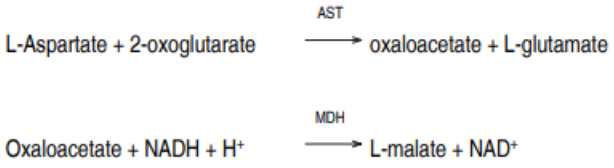
1.2. Aspartate aminotransferase (AST)

Test kit name: ASTL

Purpose

Aspartate aminotransferase, or AST, is a widely distributed enzyme in tissue, primarily in the kidney, heart, liver, and muscle. Diseases affecting these tissues are associated with elevated serum levels. Serum AST levels are also raised by hepatobiliary conditions, including viral hepatitis, metastatic cancer, and cirrhosis. Serum AST rises after myocardial infarction and peaks two days after the event (152).

The principle of AST measurement is based on the International Federation of Clinical Chemistry (IFCC) without the pyridoxal phosphate method. L-glutamate and oxaloacetate are produced when alpha-ketoglutarate and L-aspartate react, which is catalyzed by AST. The enzyme malate dehydrogenase (MDH) oxidizes NADH to NAD and changes oxaloacetate to malate. The drop in NADH absorbance at 340 nm is directly correlated with the level of AST in the blood. It is a reaction with a kinetic rate (105).



1.3.Alkaline phosphatase (ALP)

Test Kit name: ALP2

Purpose

There are four structural genotypes of serum alkaline phosphatase: intestine, placental, liver-bone-kidney, and the germ cell variety. The kidneys, spleen, placenta, prostate, small intestine, hepatocytes, leukocytes, and osteoblasts all contain the enzyme. Clinically, the liver-bone-kidney type is the most significant. In all types of cholestasis, but especially in obstructive jaundice,

there is an increase in serum alkaline phosphatase. In addition, it is increased in malignant tumors, fractures, and bone illnesses such as osteomalacia, rickets, hyperparathyroidism, and Paget's disease. Following accelerated bone growth, children and juveniles may also show large increases in serum alkaline phosphatase levels due to enhanced osteoblast activity (153).

Principles

ALP uses a straightforward reaction in which alkaline phosphatase reacts with a substrate (p-nitrophenol phosphate, or PNPP) in the presence of activators for magnesium and zinc to produce a colorful product 34 (p-nitrophenol), which is visible at 450 nm. A direct proportion is found between the quantity of alkaline phosphatase present in the samples and the rate at which p-nitrophenol is formed (106).

1.4. Total protein

Test kit name: TP2

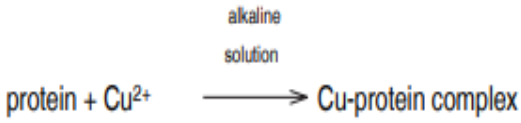
Purpose

Hypoproteinemia, or low protein levels, can result from conditions like blood loss, sprue, nephrotic syndrome, severe burns, salt retention syndrome, or Kwashiorkor. The albumin/globulin (A/G) ratio helps track the balance between these protein fractions, and significant changes can appear in liver cirrhosis, glomerulonephritis, nephrotic syndrome, hepatitis, lupus, and other acute or chronic inflammations. Measuring total protein is valuable for diagnosing and managing diseases affecting the liver, kidneys, bone marrow, or overall nutrition and metabolism.

Principles

The total protein method utilizes the biuret reaction, with measurement of the final product at 546 nm. Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the

precipitation of copper hydroxide, and potassium iodide prevents auto-reduction of copper. The color intensity is directly proportional to the protein and measured photometrically at 546nm (107).



1.5 . Albumin

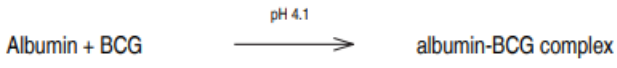
Test kit name: ALB2

Purpose

Numerous illnesses can result in hypoalbuminemia, which can be caused by several factors, including impaired synthesis from liver disease or decreased protein absorption; increased catabolism from inflammation or tissue damage (such as severe burns); malabsorption of amino acids (Crohn's disease); proteinuria from nephrotic syndrome; and protein loss through the stool (neoplastic disease). The highest plasma albumin content in severe cases of hypoalbuminemia is 2.5 g/dL. Water permeates into tissue through blood capillaries as a result of the plasma's low osmotic pressure (edema). The measurement of albumin is a great indicator of liver function and permits the tracking of a patient's dietary supplements (154).

Principles

Albumin shows a sufficiently cationic nature at pH 4.1 to form a blue-green complex with the anionic dye bromcresol green (BCG). The blue-green color intensity is measured photometrically at 570 nm and is directly proportional to the amount of albumin present in the sample (110).



1.6. Total bilirubin (TBL)

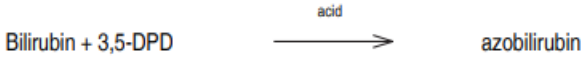
Test Kit name: BILT3

Purposes

Unconjugated (indirect) bilirubin levels rise in the bloodstream when diseases or disorders that create bilirubin more quickly than the liver can metabolize it through hemolytic mechanisms do so. Due to comparable increases in circulating unconjugated bilirubin, liver immaturity, and several other illnesses in which the bilirubin conjugation pathway is compromised. Conjugated (direct) and unconjugated (indirect) bilirubin levels in the blood rise as a result of bile duct blockage or hepatocellular injury. (155).

Principles

It worked based on the diazo reaction. Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3, 5-dichlorophenyl diazonium in a strongly acidic medium. The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically at 546 nm (108).



1.7. Direct Bilirubin (DBL)

Test kit name: BILD2

Principles

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3,5 Dichlorophenyl diazonium salt in an acid buffer to form the red-colored azobilirubin. The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically at 546 nm (109).

2. Renal function tests

2.1.Urea

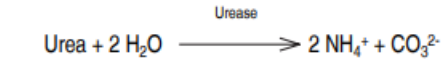
Test kit name: UREAL

Purpose

The most popular renal function screening test is BUN determination. The three forms of azotemia—prerenal, renal, and post-renal—can be differentiated with its help when combined with serum creatinine measurements. Chronic nephritis, glomerular nephritis (renal causes), nephrosclerosis, tubular necrosis, shock, reduced blood volume (prerenal causes), and urinary tract obstruction (postrenal causes) are all associated with elevated blood urea nitrogen concentrations. Temporary increases can also occur when consuming large amounts of protein. Liver illness causes levels that are unpredictable (156).

Principles

The principle of the urea work is based on the urease and glutamate dehydrogenase kinetic test. Urease hydrolyzes urea to produce carbonate and ammonium. In the second process, glutamate dehydrogenase (GLDH) and the cofactor NADH are present when 2-oxoglutarate combines with ammonium to form L-glutamate. For each mole of urea hydrolyzed in this process, two moles of NADH are oxidized to NAD+. Photometrically measured at 340 nm, the rate of decrease in the NADH concentration is directly proportional to the urea concentration in the material (111).



2.2. Creatinine

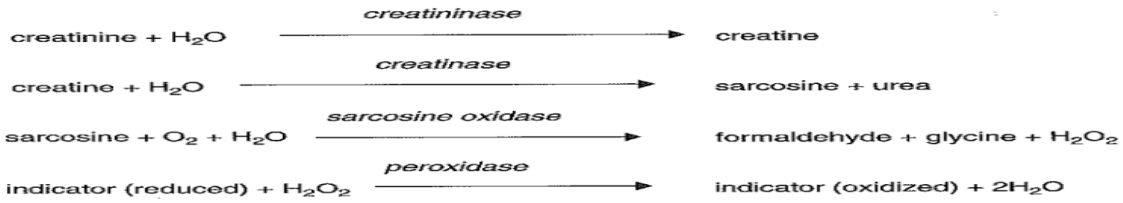
Test Kit Name: CREAP2

Purpose

The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not reabsorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted (157).

Principles

Under the action of creatinine, creatinine is converted into creatine. After that, creatinase breaks down creatine to produce urea and sarcosine. Sarcosine oxidase breaks down sarcosine into glycine and hydrogen peroxide. When peroxidase is present, the hydrogen peroxide combines with a chromophore to form a colored substance that can be measured at 546 nm (112).



2.3. Uric acid

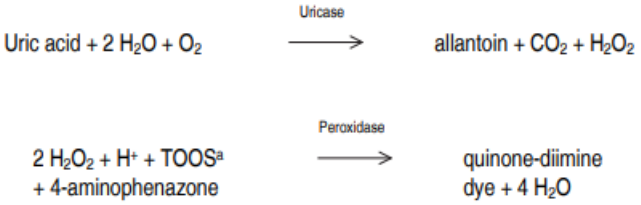
Test Kit name: UA2

Purposes

The result of purine metabolism in the human body is uric acid. Numerous renal and metabolic illnesses, such as renal failure, gout, leukemia, psoriasis, hunger or other wasting conditions, and patients on cytotoxic medications, can be diagnosed and treated with uric acid readings (158).

Principles

Uricase breaks down uric acid to produce hydrogen peroxide and allantoin. A quinone diimine dye is produced when hydrogen peroxide oxidizes 4-aminophenazone in the presence of peroxidase. 546 nm is the measurement point used in this two-point, endpoint reaction. The color intensity of the quinone diimine that is produced is measured by the increase in absorbance and is directly related to the concentration of uric acid (159).



a) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

3. Butrylcholinesterase (BChE)

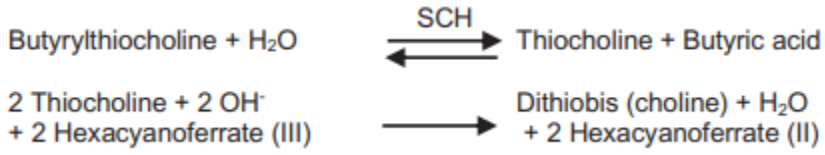
Test Kit name: CHE2

Purpose

The liver, pancreas, heart, serum, and white matter of the brain all contain cholinesterase, sometimes known as pseudo-cholinesterase or cholinesterase II. Cholinesterase's biological function is unknown. An indication of potential insecticide toxicity is serum cholinesterase. It is quantified as a liver function index. Intoxication with organophosphorus chemicals, cirrhosis, myocardial infarction, hepatitis, acute infections, and abnormal enzyme phenotypes are all associated with decreased cholinesterase levels (113).

Principles

This assay is based on the method published by Schmidt et al (113). Cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine instantaneously reduces the yellow hexacyanoferrate (III) to the almost colorless hexacyanoferrate (II). This decrease in color can be measured photometrically. The decrease in absorbance due to the conversion of hexacyanoferrate (III)into hexacyanoferrate (II), and proportional to BChE activity in the specimen, is measured at 405 nm.



Annex VI: Types of pesticides and fertilizers used at Tana Flora Floriculture Industry

About 34 pesticides were used during survey in the floriculture industry, 18 (53%) of pesticides were grouped under unlikely to cause hazard, WHO Hazard Class (Class U), but 16(43%) grouped into hazardous classes, from those 13(38%) grouped under class II, 2(6%)

class Ib and 1(3%) class III**.** Most of the active compounds belong to organophosphates, spinosyns, neonicotinoids, and morpholines**,** with widespread use of insecticides and fungicides in the horticultural sector (Table 5). The main fertilizers and acids used in the floriculture industry were: Sulphuric acid, Calcium nitrate, Ammonium phosphate, Iron, Magnesium nitrate, Potassium nitrate, Mono ammonium phosphate, Zinc sulphate, Copper sulphate, Borax, Magnesium sulphate, and Aluminum sulphate.

Types of pesticides used at Tana flora floriculture industry, Bahirdar, Ethiopia, 2025.

No	Brand Name	Active Ingredient(s)	Pesticide Group	Chemical Group / Class	WHO Hazard Class
1	Amino mite	Organosilicon 100%	Miticide	Organosilicon	U
2	Mite sweep 100EC	Hexythiazox 100 g/l	Miticide	Oxazoline	U
3	Efentizine	Clofentazine	Acaricide	Carbamate	II
4	ACE 75SP	Acephate 750 g/kg	Insecticide	Organophosphate	II
5	Orthene 97% Pellet	Acephate 970 g/kg	Insecticide	Organophosphate	II
6	Delegate 250 WG	Spinetoram 250 g/kg	Insecticide	Spinosyn	U
7	Radiant 120 SC	Spinetoram 120 g/l	Insecticide	Spinosyn	U
8	Teppeki	Flonicamid 150 g/kg	Insecticide	Pyridinecarboxamide	U
9	Tracer 485 SC	Spinosad 485 g/l	Insecticide	Spinosyn	U
10	Hinder 500 SP	Thiocyclam hydrogenoxalate 500 g/kg	Insecticide	Oxime carbamates	II
11	Belloxam 30%	Thiamethoxam 300 g/l	Insecticide	Neonicotinoid	II
12	Actara 250 WG	Thiamethoxam 250 g/kg	Insecticide	Neonicotinoid	II
13	Acepride 200 WSP	Acetamiprid 200 g/l	Insecticide	Neonicotinoid	II
14	Agent 50 SC	Fipronil 500 g/l	Insecticide	Phenylpyrazole	II
15	Echo 1.92 EC	Emamectin benzoate 192 g/l	Insecticide	Avermectin	U
16	Meltatox 385 EC	Dodemorph acetate 385 g/l	Fungicide	Morpholine	U
17	Collis 300 SC	Boscazid 200 g/l + Kresoxim methyl 100 g/l	Fungicide	Benzimidazole +SDHI	U
18	Climate 500 SC	Kresoxim methyl 500 g/l	Fungicide	SDHI	U
19	Limpulse 500 EC	Spiroxamine 500 g/l	Fungicide	Morpholine	U

20	Splendor 500 SC	Spiroxamine 500 g/l	Fungicide	Morpholine	U
21	Sprinox 500 EC	Spiroxamine 500 g/l	Fungicide	Morpholine	U
22	Sulphergold 80% WDG	Sulphur 800 g/kg	Fungicide / Miticide	Elemental Sulfur	U
23	Runner 240 SC	Methoxyfenozide 240 g/l	Insecticide	Diacylhydrazine	U
24	Match	Infenuron	Insecticide	Chloronicotinyl	II
25	Diazinox 60 EC	Diazinon 600 g/l	Insecticide	Organophosphate	Ib
26	Diazol 60 EC	Diazinon 600 g/l	Insecticide	Organophosphate	Ib
27	Agrilax	Dimethoate 40% EC	Insecticide	Organophosphate	II
28	Aliette flash	Fosetyl aluminum 800 g/kg	Fungicide	Phosphonate	U
29	Vital	Potassium phosphate	Fungicide	Phosphate compound	U
30	Previcur ESL	Propamocarb hydrochloride 66.5%	Fungicide	Carbamate	U
31	Agridif	Difenoconazole 250 g/l	Fungicide	Triazole	II
32	Jafromax	Difenoconazole 250 g/l	Fungicide	Triazole	II
33	Glyphosate	Glyphosate	Herbicide	Organophosphonate	III
34	Malathion	Malathion	Insecticide	Organophosphate	II

Note: Ia = Extremely hazardous ,Ib = Highly hazardous , II = moderately hazardous, III = slightly hazardous , U = Unlikely to present acute hazard

Annex VII: Laboratory Data Collection Sheet

A. Checklist for laboratory results

1. Liver function tests

Id. No	Parameters							
	ALT (U/L)	AST(U/L)	ALP(U/L)	DBL(mg/dl)	TBL(mg/dl)	Total protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)
Case								
PE-001								
PE- 002								
PE-003								
PE-004								
PE-005								
PE-006								
PE-007								
...								
PE-103								
Control								
NPE- 001								
NPE- 002								
NPE- 003								
NPE- 004								
NPE- 005								
NPE- 006								
NPE- 007								
…								
NPE-51								

2. Renal function tests and Butrylcholinesterase

Id. No	Parameters						
	Urea (mg/dl)	BUN (mg/dl)	Uric acid(mg/dl)	Creatinine (mg/dl)	eGFR (CKD-EPI 2021)	BCHE (U/L)	BUN to creatinine ratio
Case							
PE-001							
PE-002							
PE-003							
PE-004							
PE-005							
PE-006							
PE-007							
...							
PE-103							
Control							
NPE- 001							
NPE- 002							
NPE- 003							
NPE- 004							
NPE- 005							
NPE- 006							
NPE- 007							
…							
NPE-51							

Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: **Habtamu Molla (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Mistire Wolde (PhD, prof)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Abebe Edao (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Gobena Dedefo (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

Advisor: **Mekdes Alem (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia