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**Original Article: Open access**

# Determinates of Mortality among Adult TB/HIV Co-infected Patients in East and West Gojjam Zone, Ethiopia: A retrospective cohort study

Demelash Abewa [1,*] Endeshew Kassa [2] and Kumneger Nigussie [3]
[1] Department of Bio Statistics, Debre Markos University, Debre Markos, Ethiopia
[2] Departments of Mathematics, Debre Markos University, Debre Markos, Ethiopia
[3] Departments of Nursing, Debre Markos University, Debre Markos, Ethiopia
***Corresponding author:** E-mail: demestat21@gmail.com
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**Abstract**

The global impact of the converging dual epidemics of TB and HIV remains as one of the major public health problems. The factors have been identified that contribute to high mortality rate and low survival time of TB/HIV co-infected patients, including age, weight, AIDS staging, TB clinical presentation and calendar year. But there have been limited clinical, behavioral and socio-demographic data regarding mortality rates among HIV/TB co-infected patients and the impact of antiretroviral therapy (ART) on clinical outcomes in developing countries like Ethiopia. The present study was conducted to assess the mortality rate and the determinants of mortality of TB/HIV co-infected patients. A retrospective cohort study design was employed to retrieve relevant information from medical records to address the objectives of the study. Those TB/HIV positive patients who started ART from September, 2014 to February, 2018 in Debre Markos and Finote Selam referral hospitals were included in this study. Data were analyzed using a logistic regression model to identify the predictor variables. A total of 180 TB/HIV co-infected patients were enrolled in the present study. Of these, 40 (22.22%) patients died. Male patients, patients who respond to stress, patients with opportunistic infection, patients with low baseline CD4 count were significant predictors for increased risk of death in TB/HIV co-infected patients( $p<0.05$). Patients in WHO clinical stage I, patients in WHO clinical stage II, patients in WHO clinical stage II, patients who disclosed their TB/HIV status to the nearest person, patients whose TB were first line regimen had significant protective benefit against risk of death in TB/HIV co-infected patients ($P<0.05$). This study revealed the problem of high mortality rate in adult TB-HIV co-infected patients. It is evident that patients who are male,

advanced WHO clinical stage, low CD4, bedridden, opportunistic infection, response to stress, second line Regime and not disclosed their TB/HIV status to nearest person 1have a significant effect on survival status of patients. Health bureaus and physicians are expected to work hard to bring about behavioral changes and health education should be given to the community, the impact of stress, not disclosed, their TB/HIV status to the nearest person, and poor appetite of TB/HIV co-infected patients.

**Keywords:** Tuberculosis; Human Immunodeficiency Virus; survival status; logistic regression

## Introduction

Human Immunodeficiency Virus (HIV) and tuberculosis are commonly called the "dead duo" because HIV weakens the body's defense system, making people more prone to developing TB. People with HIV are up to 50 times more likely to develop TB in a given year than HIV-negative people [1]. Tuberculosis bacteria accelerate the progression of HIV to AIDS as a result TB is the leading cause of death for people with HIV. Globally, an estimated 13 percent of TB cases are co-infected with HIV [2]. Without proper treatment, 90% of people living with HIV die within months of contracting TB [3].

The global impact of the converging dual epidemics of TB and HIV remains as one of the major public health problems. According to the UNAIDS annual report 2009, one third of people living with HIV are co-infected with TB. Globally, about 11% of new adult cases of tuberculosis are HIV/AIDS co-infected where in sub-Saharan Africa about 31% of new tuberculosis cases are HIV/AIDS co-infected cases [4]. In particular, WHO reported that 9.27 million of the estimated new cases of TB occurred in 2007 of which 31% are in Sub-Saharan Africa and about 1.37 million (14.8%) of these being among People Living With Human Immunodeficiency Virus (PLWHIV) [5]. In general, the majority of these cases, 79% HIV positive TB incident cases, occurred in Africa in 2007. In the same year, there were 456,000 TB-related deaths among HIV-positive patients accounting for 23% of the global HIV/AIDS mortality [6].

Ethiopia is one among Africa countries most heavily affected by HIV and TB co-infection. The World Health Organization ranked Ethiopia as 7$^{th}$ among the 22 high burden countries of TB with the estimated annual incidence of 379 cases and a prevalence of 643 cases per 100,000

population [7] and the prevalence of HIV among TB patients is up to 41% [8,9]. The high burden of TB in Ethiopia might in part be attributed to the rapid increase of HIV infection, because available data indicate that HIV/ AIDS accounted for an estimated 32% or 141,000 total TB cases in 2005 [10]. According to the national annual performance report of Ethiopia, 70% of TB patients with HIV were put on ART [11], but the mortality in TB/HIV co-infected patients remains high even after starting ART. There are different factors affecting the survival of TB/HIV co-infection. Factors have been identified that contribute to high mortality rate and low survival time, including age, weight, AIDS staging, TB clinical presentation, and calendar year. However, there have been limited clinical, behavioral, and socio-demographic data regarding mortality rates among HIV/TB co-infected patients and the impact of antiretroviral therapy (ART) on clinical outcomes in developing countries like Ethiopia. Particularly, the HIV/TB co-infection data and associated factors were not assessed in the study area. Identifying these factors would help to provide better medical care to the patients. Therefore, this study was conducted to assess the mortality rate and the determinants of mortality of TB/HIV co-infected patients in the study area.

## Materials and Methods

### Study area description

The study was conducted in Debre Markos and Finote Selam referral hospitals, Amhara Regional State of Ethiopia. Debre Markos is the administrative town of East Gojjam Zone. Finote Selam is the administrative town of West Gojjam Zone. Both the two towns are found in the North West part of Ethiopia.

### Study design

A retrospective cohort study design was employed to retrieve relevant information from medical records to address the objectives of the study. Those TB/HIV positive patients who were 18 years and above and who started ART from September 01, 2014 to February 01, 2018 in Debre Markos and Finote Selam referral hospitals were included in this study. The patients' antiretroviral therapy identification numbers were used to extract the necessary information from the difference ART recording formats.

### Source of Data

The data for the present study was obtained from ART clinics in Debre Markos and Finote Selam referral hospitals. The two hospitals serve as a teaching and referral

centre for the population of East and West Gojjam zone. The hospitals have a separate unit for the ART program. Debre Markos and Finote Selam hospitals launched ART and TB treatment services in July 2003 for people living with HIV/AIDS. The hospitals started to register and give free antiretroviral therapy (ART) service for HIV infected patients who fulfill standard diagnostic criteria.

The current study reviewed patients' intake forms and follow up cards of TB/HIV patients taking anti TB and antiretroviral therapy in Debre Markos and Finote Selam referral hospitals ART clinic. The patients' charts include patient intake forms and follow-up cards, which are prepared by the Federal Ministry of Health (FMOH) to be uniformly used by clinicians to simply identify clinical and laboratory variables.

**Population frame and procedures**

All adults aged 18 years and above who followed TB-HIV care in Debre Markos and Finote Selam referral hospitals were the population. The target population for this study was patients under the follow-up of anti-TB and ART at the two referral hospitals. All TB/HIV co-infected patients of the two hospitals diagnosed between September 01, 2014 to February 01, 2018, and who fulfilled the eligibility criteria were included in the study. During the data collection procedures, patients who are transferred to other health institutions, accident dead, and any non-TB/HIV malignancies were excluded from this study. Incomplete records were also excluded from the current study. .

**Measurement of variables**

Death due to TB/HIV co-infection was the event. Age, gender, religion, educational level, response to stress, marital status, residence, past opportunistic infection, disclose of the paients status to the nearest person, functional status, baseline CD4 cell count, TB status of the patient, anemia status of the patient, baseline weight, appetite of the patient, baseline hemoglobin level, regiment of the patients, ART adherence of the patient, WHO clinical stage, alcohol drink and substance use were an independent variables.

**Data collection**

Nurses who worked in the clinics were selected to collect data from August, 2018 to November, 2018. A structured form was used to collect data on socio demographic, behavioral, and clinical factors. Lab requests, follow-up forms, ART intake forms, and patient cards were reviewed. Patients' laboratory and clinical results recorded before starting ART were used as

base line values. If there was no pre-treatment laboratory test and clinical results, however, results obtained within one month of ART initiation were considered as baseline values. Data quality was certain by using a pre-tested data collection tool and trained data collectors. Three professionals were engaged in continuous supervise, completeness and consistency of data was checked by supervisors, data coders, and investigators before and after data entry by using statistical software.

**Binomial logistic regression**

There were many situations in which the response of interest was dichotomous rather than continuous. The response variable in the present study was survival after knowledge of being TB/HIV co-infected (alive or died). To do this, the researchers used a technique known as logistic regression.

Logistic regression analysis extends the techniques of multiple regression analysis to research situations in which the outcome variable is categorical. Then the conditional probability that the patient is died given the X set of predictor variables is denoted by Prob (Yi =1 | X) =Pi. The expression Pi has the form:

$$P_i = \frac{e^{(\beta_0+\beta_1 X_{1i}+\beta_2 X_{2i}+----+\beta_r X_{ri})}}{1+e^{(\beta_0+\beta_1 X_{1i}+\beta_2 X_{2i}+----+\beta_r X_{ri})}} = \frac{e^{X'\beta}}{1+e^{X'\beta}}$$

Pi = the probability of the i$^{th}$ patient died due to TB/HIV

Yi = observed survival status of patient TB/HIV co-infected patient i

β is a vector of unknown coefficients.

**Statistical analysis**

The data was coded and entered with Epi data 3.1 for Windows. Then export to STATA version 14. Chi square test was used to test the relationship between each covariate and survival status TB/HIV co-infection patients. Multi covariate logistic regression was done by entering all variables with *P* value less than 0.25 in the univariate (chi-square) analysis. Finally, logistic regression with forward likelihood selection method with *P* value less than 0.05 was used to identify the determinants of mortality in TB/HIV.

Prob (Yi =1 | X) =Pi. The expression Pi has the form:

**Ethics approval and consent to participate**

Ethical clearance was obtained from Debre Markos University, College of Natural and Computational Science Research Coordinator office. To care for patient secrecy, nurses working in the clinics

extracted the data from patients' medical records. Moreover, no personal identifier was used on the questionnaire.

**Results**

**Socio-demographic characteristics of TB and HIV co –infected patients**

There were 180 TB/HIV co-infected patients in this study. Of these, 95 (52.8%) were male patients while 85 (47.2%) were female patients. Patients with no formal education accounted for the larger proportion 74 (41.1%) compared to those with primary education 53(29.4%), secondary education/ preparatory 34(18.9%), and college and above 19 (10.6%). The majority of TB/HIV patients 108(60%) were urban residences while 72 (40%) were residing in rural areas. The larger proportion of TB/HIV patients 170 (94.4%) were Orthodox religion followed while 10(5.6%) were following Muslim religion. TB/HIV Patients whose marital status single accounted for a larger proportion 108 (60%) compared to those with married 41(22.8%) and divorce 31(17.2%) (Table1).

Table 1 Socio-demographic characteristics of TB/HIV patients

| variable | Category | Frequency | Percent |
|---|---|---|---|
| Gender | Male | 95 | 52.8 |
| | Female | 85 | 47.2 |
| Education level | No education | 74 | 41.1 |
| | Primary | 53 | 29.4 |
| | Secondary/ preparatory | 34 | 18.9 |
| | College and above | 19 | 10.6 |
| Religion | Orthodox | 170 | 94.4 |
| | Muslim | 10 | 5.6 |
| Marital status | Married | 41 | 22.8 |
| | Single | 108 | 60 |
| | Divorce | 31 | 17.2 |
| Residence | Urban | 108 | 60 |
| | Rural | 72 | 40 |

**Clinical and behavioral characteristics of TB and HIV co –infected patients**

Patients with work baseline functional status accounted for the larger proportion 99 (55%) compared to those with ambulatory baseline functional status 62(34.4%) and bedridden baseline functional status 19(10.6%).The majority of participants who had TB/HIV co-infected 73(40.6%) were in WHO clinical stage II followed by WHO clinical stage III, 49 (27.2%), WHO clinical stage I 47 (26.1%) and WHO clinical stage IV 11 (6.1%) respectively. Among TB/HIV co-infected patients, 165 (91.7%) were non-smokers, 129 (71.7%) were not taking alcohol, 153 (85%) were first line regiment, 119 (66.1%) had poor appetite and 85 (47.2%) had stress. The majority of TB/HIV co-infected patients 118 (65.6%) were disclosed their TB/HIV status to the nearest person while 62 (34.4%) were not disclosed their TB/HIV status to the nearest person. Among TB/HIV co- infected patients, patients with pulmonary TB 165(91.7%) higher than extra pulmonary TB 15(8.3%) (Table 2).

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Table 2 Clinical and behavioral characteristics of TB/HIV patients

| Variable | Category | Frequency | Percent |
|---|---|---|---|
| Functional status of the patient | Work | 99 | 55 |
| | Ambulatory | 62 | 34.4 |
| | Bedridden | 19 | 10.6 |
| Baseline WHO clinical stage | I | 47 | 26.1 |
| | II | 73 | 40.6 |
| | III | 49 | 27.2 |
| | IV | 11 | 6.1 |
| Regiment of the patients | First line | 153 | 85 |
| | Second line | 27 | 15 |
| Disclosed about your status to the nearest | Yes | 118 | 65.6 |
| person | No | 62 | 34.4 |
| TB status of the patient | Pulmonary | 165 | 91.7 |
| | Extra pulmonary | 15 | 8.3 |
| Anemia status of the patient | Normal | 122 | 67.8 |
| | Mild | 48 | 26.7 |
| | Moderate | 10 | 5.6 |
| ART adherence of the patient | Good | 122 | 67.8 |
| | Fair | 34 | 18.9 |
| | Poor | 24 | 13.3 |
| Tobacco smoke | Yes | 15 | 8.3 |
| | No | 165 | 91.7 |
| Response of stress | Yes | 85 | 47.2 |
| | No | 95 | 52.8 |
| Alcohol drink | Yes | 129 | 71.7 |
| | No | 51 | 28.3 |
| appetite of the patient | Good | 61 | 33.9 |
| | Poor | 119 | 66.1 |

In the year September 01, 2014 to February 01, 2018 follow-up period, patients who fulfilled the inclusion criteria of this study, a total of 40 (22.22%) TB and HIV co-infected patients were died and the remaining (140) 77.78% TB and HIV co - infected patients were alive (Figure 1).

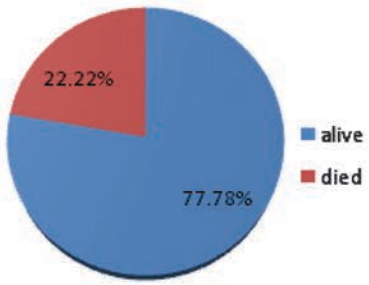


Figure 1 survival status of the patients in the study area

The average baseline age of TB and HIV co-infected patients was 33.6611 years with a minimum 18 years and maximum 60 years. The average baseline CD4 cell count and hemoglobin levels of TB and HIV co-infected adult patients in the study area was 196.87 cells/µl and 11.94 mg/dl respectively. The TB and HIV positive adult patients in the study area had an average baseline weight and BMI of 50.72kg and 19.305kg/$m^2$ respectively (Table 3).

Table 3: description of continuous covariates for TB and HIV co-infected patients

| No | Covariate | mean | Std.Dev. | Minimum | Maximum |
|---|---|---|---|---|---|
| 1 | Baseline age | 33.66 | 9.07 | 18 | 60 |
| 2 | Baseline CD4 count | 196.87 | 169.19 | 6 | 1105 |
| 3 | Baseline hemoglobin | 11.94 | 2.03 | 6.8 | 22.7 |
| 4 | Baseline weight | 50.72 | 9.25 | 30 | 80 |
| 5 | Baseline BMI | 19.31 | 2.53 | 10.9 | 29.6 |

The proportion of male respondents who had TB/HIV-co infection accounted for a larger proportion of deaths in the sample (70%) compared to female respondents (30%). Patients with ambulatory accounted for the larger proportion (52.5%) of deaths compared to those with bedridden (35%), and working functional status (12.5%). The majority of deaths in TB/HIV patients (77.5%) were response to stress while 22.5% were not response to stress. Among TB/HIV co-infected patients' deaths, 85% were non-smokers and 82.5% of TB/HIV co-infected patients deaths were poor appetite. The majority of participants who died from TB/HIV co-infection, 40% were in WHO clinical stage III followed by WHO clinical stage II (25%) and WHO clinical stage IV (22.5%). Only (12.5%) study participants who died from TB/HIV-co

infection were found to be in WHO clinical stage I.

Chi-square test shows that mortality in TB/HIV co infection was significantly associated with gender of the patient, baseline WHO clinical stage, baseline functional status, regimen of the patient, tobacco smoke, response to stress, appetite of the patient, disclosing your status to the nearest person and baseline opportunistic infection. Multi-variate logistic regression was done by entering all variables with *P* value less than 0.25 in the univariate (chi-square) analysis (Table 4).

Table 4: The association between socio- demographic, clinical, behavioral characteristics and survival status of the patients

| | | Survival status of the patients | | | |
|---|---|---|---|---|---|
| **Variables** | **Category** | **Alive, n (%)** | **Died, n (%)** | **chi-square** | **P -value** |
| WHO clinical stage | Stage I | 42(30) | 5(12.5) | | |
| | Stage II | 63(45) | 10(25) | | |
| | Stage III | 33(23.6) | 16(40) | 32.406 | <0.001* |
| | Stage IV | 2(1.4) | 9(22.5) | | |
| Functional status | Working | 94(67.1) | 5(12.5) | | |
| | Ambulatory | 41(29.3) | 21(52.5) | 50.870 | <0.001* |
| | Bedridden | 5(3.6) | 14(35) | | |
| Gender | Male | 67(47.9) | 28(70) | 6.12 | 0.019* |
| | Female | 73(52.1) | 12(30) | | |
| Regimen of the patient | First line | 126(90) | 27(67.5) | 12.353 | 0.002* |
| | Second line | 14(10) | 13(32.5) | | |
| Tobacco smoke | yes | 9(6.4) | 6(15) | 2.992 | 0.104* |
| | No | 131(93.6) | 34(85) | | |
| Response to stress | Yes | 54(38.6) | 31(77.5) | 18.917 | <0.001* |
| | No | 56(61.4) | 9(22.5) | | |
| appetite of the patient | Good | 54(38.6) | 7(17.5) | 6.166 | 0.014* |
| | Poor | 86(61.4) | 33(82.5) | | |
| disclosed your status to the nearest person | Yes | 106(75.7) | 12(30) | 28.793 | <0.001* |
| | No | 34(24.3) | 28(70) | | |
| opportunistic infection | Yes | 37(26.4) | 31(77.5) | 34.521 | <0.001* |
| | No | 103(73.6) | 9(22.5) | | |

*The relationship is significance at $\alpha = 0.25$

**Predictors of mortality in TB-HIV co-infected patients**

From the nominated 11 variables for multiple covariate logistic regression, about eight variables passed the filtration of forward stepwise LR of logistic regression. Namely, Gender, WHO clinical stage, Functional status, CD4 count, Opportunistic infection, Response to stress, Regimen and Disclosed your status to nearest person have a significant effect on the survival status of patients ($\alpha$=0.05). Contrarily, variables such as tobacco, appetite and hemoglobin level have less effect on survival status of TB/HIV co-infected patients.

After adjusting other covariates, male TB/HIV patients were 8.045 more likely to die than the reference category (female). TB/HIV Patients in WHO clinical stage I were 96.8% less likely to die than the patients in WHO clinical stage IV (AOR=0.032, 95%CI=0.003, 0.377). Similarly, patients in WHO clinical stage II were about 97.2% less likely to die than those patients in WHO clinical stage IV (AOR=0.022, 95%CI=0.002, 0.241). Patients in WHO clinical stage III were 92.3% less likely to die than that of the patients in WHO clinical stage IV (AOR=0.077, 95%CI=0.008, 0.749). TB/HIV Patients with working functional status were 92% less likely (AOR=0.08, 95%CI=0.012, 0.533) to die than patients recorded as being in ambulatory functional status. TB/HIV patients' who exposed their TB/HIV status to the nearest person were 80.7% less likely (AOR=0.193, 95% CI=0.048, 0.782) to die than patients who do not expose their TB/HIV status to the nearest person. TB/HIV patients who had stress were 6.365 times more likely to die than TB/HIV patients' who had no stress. TB/HIV co-infected patients with opportunistic infection were 8.831 times more likely (AOR=8.831, 95%CI=2.239, 34.833) to die than patients without opportunistic infection. Patients' CD4 T-lymphocytes count increase by one unite, the odds of mortality decreased by 0.9% (AOR=0.991, 95%CI=0.983, 0.999) (Table 5).

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Table 5: Parameter Estimated Odds Ratio for the Final Logistic regression

| Covariates | Category | AOR (95% CI) | P –value |
|---|---|---|---|
| Gender | Male | 8.045 (1.767, 36.623) | 0.007* |
| | Female | 1 | |
| | I | 0.032 (0.003, 0.377) | 0.006* |
| WHO clinical stage | II | 0.022(0.002, 0.241) | 0.002* |
| | III | 0.077(0.008, 0.749 | 0.027* |
| | IV | 1 | |
| Functional status | Working | 0.080(0.012, 0.533) | 0.009* |
| | Ambulatory | 0.502(0.098, 2.583) | 0.410 |
| | Bedridden | 1 | |
| CD4 count | | 0.991 (0.983, 0.999) | 0.032* |
| Opportunistic infection | Yes | 8.831 (2.239, 34.833) | 0.002* |
| | No | 1 | |
| Response to stress | Yes | 6.365(1.511, 26.815) | 0.012* |
| | No | 1 | |
| Regimen | First line | 0.204(0.043, 0.960) | 0.044* |
| | Second line | 1 | |
| Disclosed your status to nearest person | Yes | 0.193(0.048, 0.782) | 0.021* |
| | No | 1 | |

*Odds ratio is significance at alpha=0.05

**Discussion**

The rate of mortality in TB/HIV co-infection was moderately high at 22.22%. This was in line with other studies done in Northwest Ethiopia [12]. It was lower than 43.1% reported from Barcelona, Spain [13]. The findings of this study were higher compared to studies conducted in Southwest Ethiopia (20.2%), Ambo Ethiopia (15.8%), Eastern Ethiopia (11.1%), and Ethiopia [15-18]. These wide variations in the mortality rate of TB/HIV co infected patients across the globe, as reported, can partly be accounted for the following reasons: coverage level of highly active antiretroviral treatment, under reporting, different sample size and study methodology applied.

The male TB/HIV co-infected patients were 8.045 times more likely to die than female TB/HIV co-infected patients. This was in line with the study done in Ethiopia[17], but in contrast with the retrospective study done in Brazil [18] and Southwest Ethiopia [14].

The reason it contradicts with a study in Brazil and Jimma would be due to personal differences and occupational status might decrease the survival time of the patients.

TB/HIV patients with working functional status were 92% less likely to die than patients recorded as being in ambulatory functional status. A study conducted in Southwest Ethiopia [14], Ambo, Ethiopia[15] and Northwest Ethiopia [12] revealed a similar findings where bedridden functional status was the risk factor for mortality in TB/HIV co-infected patients. The other important finding identified was the association of patient's WHO clinical stage with the mortality TB-HIV co infection patients. Those patients in first, second, and third WHO clinical stages were about 96.8%, 97.2 % and 92.3% less likely to die compared to those in WHO clinical stage IV, respectively. This result is consistent with the study conducted in Ambo, Ethiopia[16], Eastern Ethiopia [16], and Nigeria [19]. In contrast, a study from southwest Ethiopia [14] has shown that patients in WHO stages 2, 3, and 4 compared to stage 1 were found protective for mortality risk.

The results of the present study showed that patients' CD4 T- lymphocyte count increase by one unite, odd of mortality decreased by 0.9% (OR=0.991, 95%CI=0.983-0.999). The impact of CD4 cell count on the survival status of TB/HIV Co-Infected has been assessed by several studies, the findings of which showed that the depletion of CD4 cell count was associated with high risk of death. A retrospective cohort study conducted in Barcelona, Spain, showed that survival was worst among patients with <200/mm3 CD4 [13]. A study conducted in Pernambuco, Brazil, indicated that CD4 count <200/mm3 was a significant risk factor of death of HIV-TB co infected patients [18]. A study conducted in Ambo Referral Hospital, Ethiopia [15] and Northwest Ethiopia [12] revealed the same result to the present study. TB/HIV co-infected patients with opportunistic infection were 8.831 times more likely to die than patients without opportunistic infection. The possible reason would be opportunistic diseases are a heterogeneous group of diseases, infections, and malignancies that result in significant morbidity and mortality in TB-HIV co-infected individuals. Progressive deterioration of the immune system caused by a decline in CD4 + T cells is the main

risk factor for the development of opportunistic diseases and it leads to death.

TB/HIV co-infected patients with stress were 6.365 times more likely to die than patients without any stress. This finding is in line with a study conducted in Oromia regional state, Ethiopia, showing that self-stigma or being discriminated was associated with a poor quality of life in the psychological domain [20]. The possible reason would be if TB/HIV co-infected patients have ever felt stressed out , being under pressure can affect their body, either by causing headache, muscle tightness, or flutters in the patient chest; making TB/HIV patients feel down in the dumps; or leaving you ravenous for chocolate or robbed of all appetite that accelerated the risk of death. The TB/HIV patients who exposed their TB/HIV status to the nearest person were 80.7% less likely to die than patients who did not expose their TB/HIV status to the nearest person. In contrast, a study from southwest Ethiopia found that exposing their TB/HIV status to the nearest person was not associated with death in TB/HIV co infected patients [14]. This difference could be due to the high prevalence of patients exposing their TB/HIV status to the nearest person in in the present study population.

## Conclusion

This study revealed the problem of high mortality rate in adult TB-HIV co-infected patients. Results from this study demonstrated that male patients, advanced WHO clinical stage (WHO clinical III and IV), low baseline CD4 cell count, bedridden patients, having opportunistic infection, patient response to stress, second line regimen and disclosed your TB/HIV status to the nearest person were found to be highly associated with mortality of TB/HIV patients.

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# Laboratory Evaluation of Botanicals extracts against Pea Weevils *Bruchus pisorum* L. (Coleoptera: Bruchidae)

Cherinet Ayele[1] and Getnet Atenafu[1]

[1]*Department of Biology, College of Natural Sciences, Debre Markos University, Ethiopia*
**Corresponding Author**: Getnet Atenafu, Email: getnet.atenafu@gmail.com

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## Abstract

The botanical extracts degrades more rapidly than most synthetic pesticides, and considered relatively environment friendly with less likely to kill beneficial pests than chemical synthetic pesticides. The aim of the present study was to evaluate the efficacy of aqueous and ethanolic extracts of *Zingiber officinale*, *Brassica nigra*, *Phytolacca dodecandra*, *Nicotiana tobacum* and *Allium sativum* against adult *Bruchus pisorum* under laboratory conditions. Each of the extracts was tested separately and in combinations for the synergistic effects against the target insect. The experiment designed in completely randomized design with three replications. Deltamethrin dust and untreated control were included in the treatment. The extract powders of 0.07%, 0.12% and 0.17% w/w of each plant component were mixed thoroughly with 100 gram grains in each treatment cup. The experiment was conducted in 2019/2020. The F1 progeny laboratory reared 20 adults were released into the treatment jars. Number of dead weevils, weight loss, and number of germinated grains were recorded after treatment application. Toxicities level of botanical extracts against *Bruchus pisorum* were different and the aqueous extracts provided the highest efficacy. Among the five selected botanicals, aqueous extract of *P. dodecandra* leaf powder caused 100% mortality by 0.17% w/w concentration of 72 hours after treatment application. Whereas ethanol extracts were the least effective compared to the rest of the botanicals and standard check, but significantly better than untreated check ($P<0.05$). Insect mortality was directly proportional with the dose administered and significant differences were observed among doses. The present study demonstrated that the crude

extracts of the botanicals have no effect on seed germination after the treatment. It could be concluded that the extract of *P. dodecandra* to be an alternative for the control of *Bruchus pisorum*. Further investigation on the fractionation of the bioactive components of botanicals is recommended.

**Keywords**: Botanical; *Bruchus pisorum*; Germination; Mortality; *Pisum sativum*

## Introduction

Insect pests cause damage to stored grains by reducing weight, nutritional value and seed viability. A direct loss of stored grains by feeding as well as promoting colonization of damaged grains by secondary pests including other insects and fungi are causes of storage insect pests [1]. *Bruchus pisorum*, is one of the major stored pea grain pests reported in Ethiopia [2]. Pea weevils begin their attack in the field and continue during storage [3]. Larvae once hatched burrow through the pod wall and into the seed creating a small, dark entry hole approximately 0.2 mm in diameter [4].

Management of this pest is difficult because its larvae feed inside the grains, reducing the possibility to control using direct insecticidal treatments [5]. However, still the protection of stored grains from insect damage relies on applying synthetic pesticides, such as fumigation with phosphine or methyl bromide, or Deltamethrin dust [6]. Searching for alternatives for the chemical pesticides is the current focus of our research. Therefore, the aim of the present study was to investigate the efficacy of five selected botanical extracts against *Pisum sativum* under laboratory conditions.

## Material and Method

### Experimental design

The study was carried out at Debre Markos University, Department of Biology laboratory in 2019 to 2020.The experiments were arranged in a Completely Randomized Design (CRD) with three replications. The numbers of treatments were composed of sole and combination of five types of botanicals with three rates of application (0.07% w/w, 0.12% w/w and 0.17% w/w). Deltamethrin dust was used as standard check and untreated control also used for comparison. Each dose of the botanicals was mixed with 100g of *P. sativum*. The treatments were thoroughly mixed with *P.*

*sativum* seeds by shaking and rolling the cups gently to ensure uniform coating of the seeds. Twenty *B. pisorum* individuals of unsexed F1 progeny were released into each treatment jars following the method of [7]. After 2 hours for acclimatization, botanical extracts were mixed into each treatment jars. Percentage mortality target insect, weight loss, and germination test of grains were assessed.

**Insect culture**

Sixty five kilogram of field pea was bought from local farmers around Motta town, East Gojjam Zone of Amhara Region, Ethiopia. Field pea weevils were collected from Debre Markos town local grain stores. Insect identification was done based on the appropriate methods [8]. Before rearing the target insect, *P. sativum* was dried in an oven at 60°C for 6 hours to disinfect and allowed to cool for 2 hours according to the method described by [9]. The *B. pisorum* were reared in the three plastic jars of 5 liters capacity each containing 2 kilogram of disinfected grains. The plastic jars were washed by tap water and cleaned by ethanol before two days to start rearing. About 450 unsexed adult weevils were collected from infested grain and cultured in each jar and the jars were covered with mesh cloth and fixed with rubber bands.

The laboratory condition was kept in 28-30°c and 60-70 % relative humidity for 14 days of oviposition time and for the experiment periods. The relative humidity was maintained by boiling water with a small electric boiler at experimental room and room temperature recorded daily by using a cylindrical thermometer until the end of the experiment. After 14 days of oviposition period, the adult pea weevils were discarded from the rearing jars. The rearing jars containing eggs on the substrate (peas) were kept under laboratory condition for 28 days until the emergence of F1 progenies.

**Plant collection, identification and extraction**

The plant materials were collected from farmers' fields around Debre Markos town and identified the species by Debre Markos University Biology department experts. These botanical plants were washed with tap water to remove dust. The plants were dried for 10 days under shady conditions and then grind using pestle and mortar. The plant materials parts used for extraction were *Z. officinale* (rhizomes), *B. nigra* (seed), *P. dodecandra* leaf (leaf), *N. tobacum*) and *A. sativum* (bulbs). The solvents for extraction were water and ethanol. Sensitive balance was used to measure 25g portion of each grind fine powder to soak in 100 ml of each solvent and shacked for 24 hours by shaker to produce uniformly distributed powder particles in the solution.

Each solution supernatant was filtered by muslin cloths to remove coarse materials followed by filter paper whatman (No.1) to remove impurities. These extracts were dried in a well-ventilated area under shade covered by muslin close. Powder of these plant parts were stored and labeled in air-tight glass jars in a cool place until use for the experiment.

**Adult mortality test**

Based on the experimental design of the present the present study, the mortality effect of 0.07%, 0.12%, and 0.17% w/w of each botanical and their combinations were evaluated. After 24, 48, and 72 hours of treatment application, each 20 unsexed laboratory reared adult weevils of the same age group were observed their mortality. gfgvv samples were taken from each jar at random after three days of the target insect mortality test. The number of damaged grains, which has hole, and undamaged grains counted and weighed and the estimated percentage weight loss was calculated by using the method described by Nikolova [10].

Percentage weights losses = $\frac{(Wu \times Nd) - (Wd \times Nu)}{Wu \times (Nd + Nu)} \times 100$

Where: Wμ = weight of undamaged grains in $n^{th}$ sample of N, and Nμ = number of undamaged grains; Wd = weight of damaged grains; Nd = number of damaged grains.

**Germination Test Assay**

Germination assay was carried out at the end of three days efficacy test. Fifty grain samples were taken at random from each replication of all the treatments and the placed in Petri dishes contained moist filter paper (Whatman No 1). The germinated seeds emerged from each Petri dish were counted and recorded after five days. Percent of germination were calculated by using the method describes by [11].

Percentage germination (%) $= \frac{NG}{TG} \times 100$

Where NG = number of germinate seeds
TG = total number of grain samples test in each petridish.

Data Analysis

Data analysis was done by using Microsoft Excel and SPSS Version 25. One-way analysis of variance (ANOVA) was used to analyse effects of botanicals treatment, percentage of mortality, weight loss, and germination of field pea grains. Mean comparisons were conducted using Tukey's Studentized range test (HSD) at 5% level of significance ($p<0.05$). Probity analysis was applied to determine lethal dosages causing 50% ($LC_{50}$), 90% ($LC_{90}$) mortality of *B. pisorum*.

## Results and Discussion

### Effect of botanical extracts against *Bruchus pisorum*

Mortality of adult *B. pisorum* due to individual botanical aqueous extracts with different concentrations is presented in table1. Among the botanicals, *P. dodecandra* at 0.17%w/w concentration caused the highest (100%), mortality, but *N. tobacum* resulted the lowest (75%) mortality at 72 hours exposure times at concentration of 0.17 %w/w in 72 hours exposure time. The present study is in line with Qwarse *et al.* (2016), where leaf extracts of *P. dodecandra* at 150 mg/ml concentration caused 99% mortality *B. pisorum* after 72hr. of exposure time.

Table.1. Mean ± SE percent mortality of *B. pisorum* at different concentration rate of the individual plant aqueous extracts at 24, 48, 72 hours exposure time under laboratory condition.

| Treatment | Dose (w/w) | Mean ± SE adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *Allium sativum* | 0.07 | 13.3±0.33$^{b}$ | 25.00±0.57$^{b}$ | 46.66 ±0.33$^{c}$ |
| | 0.12 | 28.3±0.88$^{a}$ | 60.00±0.00$^{a}$ | 71.66±0.33$^{b}$ |
| | 0.17 | 26.6±1.20$^{a}$ | 65.00±0.33$^{a}$ | 88.33±0.33$^{ab}$ |
| *Zingeber officinal* | 0.07 | 20.0±0.57$^{b}$ | 35.00±0.00$^{b}$ | 50.00±0.33$^{b}$ |
| | 0.12 | 25.0±1.00$^{a}$ | 51.66±1.76$^{a}$ | 75.00±1.15$^{ab}$ |
| | 0.17 | 30.0±0.57$^{a}$ | 63.33±0.33$^{a}$ | 90.00±1.00$^{ab}$ |
| *Brassica nigra* | 0.07 | 10.0±0.00$^{b}$ | 23.330.33$^{c}$ | 43.33±0.88$^{c}$ |
| | 0.12 | 18.3±1.20$^{b}$ | 38.33±0.66$^{b}$ | 48.33±1.20$^{c}$ |
| | 0.17 | 26.7±1.20$^{a}$ | 55.00±1.15$^{a}$ | 75.00±0.57$^{ab}$ |
| *Nicotiana tobacum* | 0.07 | 20.0±0.57$^{b}$ | 38.33±0.88$^{b}$ | 61.66±0.66$^{b}$ |
| | 0.12 | 28.3±1.45$^{a}$ | 61.67±0.33$^{a}$ | 80.00±0.57$^{ab}$ |
| | 0.17 | 30.0±1.00$^{a}$ | 68.33±0.66$^{a}$ | 91.33±0.33$^{ab}$ |
| *Phytolacca dodecandra* | 0.07 | 20.0±1.00$^{b}$ | 43.33±1.20$^{b}$ | 71.66±0.33$^{b}$ |
| | 0.12 | 26.7±0.88$^{a}$ | 58.33±0.33$^{a}$ | 81.66±0.66$^{ab}$ |
| | 0.17 | 28.3±0.88$^{a}$ | 68.33±0.88$^{a}$ | 100.00±0.00a |

Means followed by the same letter within column are not significantly different at 5% level, HSD

Percent mortality of *B. pisorum* at different rate combinations of two plant aqueous extracts is presented in table 2. The combination of *N. tobacum* and *P. dodecandra* of 0.17w/w% concentration caused the highest mortality (91.66%) whereas the lowest (75%) was recorded by the combination of *A. sativum* and *B. nigra* against *B. pisorum* at 72 hours' time of exposure. The result showed that *P.dodecandra* is more toxic to *B. pisorum* than the other aqueous extracts. It might be due to the difference in the nature of the bioactive compounds. Qwarse, [12] also

reported that the toxic effect of *P. dodecandra* against bruchids might be due to the presence of saponin, alkaloid, sterol, triterpenoids, phenol, flavonoid and glycoside. Studies also revealed that the insecticidal effect of plant powder may contribute to repellency, a stomach poisoning effect where insects feed on admixed grain and pick up a lethal dose of treatment particles. And these powders might be in a situation to reduce insect movement and also cause death through occlusion of their spiracle, thereby, preventing respiration via trachea [13].

Table .2. Mean ±SE percent mortality of *B. pisorum* at different rate of the combination of two plant aqueous extracts at 24, 48, 72 hours exposure time.

| Treatment | Dose (w/w) | Mean±SE adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *A. sativum* and *Z. officinal* | 0.07 | 13.33±0.33$^{b}$ | 33.33±0.33$^{b}$ | 56.66±0.33$^{b}$ |
| | 0.12 | 26.66±0.88$^{a}$ | 55.00±1.15$^{a}$ | 70.00±1.00$^{b}$ |
| | 0.17 | 28.33±0.66$^{a}$ | 65.00±0.00$^{a}$ | 88.33±0.33$^{a}$ |
| *A. sativum* and *B. nigra* | 0.07 | 11.66±0.33$^{b}$ | 23.33±0.33$^{c}$ | 43.33±0.66$^{c}$ |
| | 0.12 | 20.00±0.57$^{b}$ | 41.66±0.33$^{b}$ | 56.66±0.66$^{b}$ |
| | 0.17 | 25.00±0.57$^{a}$ | 51.66±0.66$^{a}$ | 75.00±1.00$^{a}$ |
| *Z. officinal*and *B. nigra* | 0.07 | 15.00±0.58$^{b}$ | 30.00±0.57$^{b}$ | 51.66±0.66$^{b}$ |
| | 0.12 | 21.66±0.88$^{b}$ | 46.66±0.33$^{b}$ | 58.33±0.33$^{b}$ |
| | 0.17 | 21.67±0.66$^{b}$ | 56.67±0.88$^{a}$ | 78.33±1.45$^{b}$ |
| *A. sativum* and *P. dodecandra* | 0.07 | 16.66±0.33$^{b}$ | 38.33±0.88$^{b}$ | 63.33±0.33$^{b}$ |
| | 0.12 | 26.67±0.88$^{a}$ | 55.00±0.57$^{a}$ | 76.66±0.33$^{a}$ |
| | 0.17 | 30.00±0.57$^{a}$ | 63.33±1.20$^{a}$ | 90.00±1.52$^{a}$ |
| *Z. officinal* and *P. dodecandra* | 0.07 | 20.00±0.57$^{b}$ | 43.33±0.66$^{b}$ | 65.00±0.57$^{b}$ |
| | 0.12 | 26.66±0.88a | 58.33±0.33$^{a}$ | 76.66±0.33$^{a}$ |
| | 0.17 | 30.00±0.57$^{a}$ | 61.66±0.88$^{a}$ | 90.00±0.57$^{a}$ |
| *B. nigra* and *P.dodecandra* | 0.07 | 13.33±0.33$^{b}$ | 25.00±0.66$^{b}$ | 48.33±0.33$^{c}$ |
| | 0.12 | 20.00±0.57$^{b}$ | 43.33±0.88$^{b}$ | 61.66±0.33$^{b}$ |
| | 0.17 | 28.33±0.33$^{a}$ | 58.33±0.66$^{a}$ | 81.66±0.33$^{a}$ |
| *A. sativum* and *N. tobacum* | 0.07 | 15.00±0.57$^{b}$ | 30.00±0.57$^{b}$ | 53.33±0.88$^{b}$ |
| | 0.12 | 23.33±0.33$^{b}$ | 46.65±0.33$^{b}$ | 61.66±0.88$^{b}$ |
| | 0.17 | 26.67±0.88$^{a}$ | 60.00±1.00$^{a}$ | 81.66±0.66$^{a}$ |
| *Z. officinal* and *N. tobacum* | 0.07 | 15.00±0.57$^{b}$ | 31.66±0.33$^{b}$ | 58.33±1.20$^{b}$ |
| | 0.12 | 25.00±0.57$^{a}$ | 51.66±0.33$^{a}$ | 75.00±1.15$^{a}$ |
| | 0.17 | 28.33±0.33$^{a}$ | 65.00±0.00$^{a}$ | 90.00±0.57$^{a}$ |
| *B. nigra* and *N. tobacum* | 0.07 | 13.00±0.58$^{b}$ | 30.00±0.57$^{b}$ | 46.66±0.66$^{c}$ |
| | 0.12 | 21.66±0.88$^{b}$ | 46.66±0.66$^{b}$ | 60.00±0.57$^{b}$ |
| | 0.17 | 26.66±0.88$^{a}$ | 60.00±0.00$^{a}$ | 80.00±0.57$^{a}$ |
| *P. dodecandra* and *N. tobacum* | 0.07 | 21.66±0.33$^{b}$ | 41.66±0.66$^{b}$ | 66.66±0.88$^{b}$ |
| | 0.12 | 28.33±0.88$^{a}$ | 60.00±0.57$^{a}$ | 80.00±0.00$^{a}$ |
| | 0.17 | 30.00±0.58$^{a}$ | 66.66±0.33$^{a}$ | 91.66±0.33$^{a}$ |

Means followed by the same letter within column are not significantly different at 5% level, HSD

The combination of three plant aqueous extracts against *B. pisorum* is presented in table 3. The mixture of *Z. officinal, P. dodecandra* and *N. tobacum* showed the highest *B. pisorum* mortality (88.6%) in the concentration of 0.17w/w at 72 hours' time of exposure. But the lowest mortality (76.66%) was recorded in the combination of *A. sativum* and *B. nigra* in the concentration of 0.17w/w at 72 hours. The difference might be due the presence of *P. dodecandra* for the mortality of *B. pisorum.* Asmare [14]. Studies also reported that *P. dodecandra* caused 100% mortality to the control of weevil within 28 days of infestation.[14] It is also evident that storage crop protections are supported by the use of botanicals like *P. dodecandra* in many African countries [15].

Table 3 Mean ±SE percent mortality of *B. pisorum* at different rate with the combination of the three plant aqueous extracts at 24, 48, 72 hours exposure time

| Treatment | Dose (w/w) | Mean ± SE adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *A. sativum, Z. officinal* and *B. nigra* | 0.07 | 13.33±0.33$^{c}$ | 25.00±0.00$^{c}$ | 45.00±0.57$^{c}$ |
| | 0.12 | 21.66±0.66$^{c}$ | 46.67±0.33$^{c}$ | 61.66±0.33$^{b}$ |
| | 0.17 | 26.66±0.33$^{b}$ | 56.66±0.88$^{b}$ | 76.66±1.45$^{a}$ |
| *A. sativum, Z. officinal* and *P.dodecandra* | 0.07 | 15.00±0.57$^{c}$ | 31.66±0.66$^{c}$ | 58.33±1.45$^{b}$ |
| | 0.12 | 26.66±0.88$^{b}$ | 58.33±0.66$^{b}$ | 75.00±0.57$^{a}$ |
| | 0.17 | 30.00±0.57$^{b}$ | 60.00±1.15$^{b}$ | 86.66±0.88$^{a}$ |
| *A. sativum, B. nigra* and *P.dodecandra* | 0.07 | 16.66±0.33$^{c}$ | 31.33±0.88$^{c}$ | 55.00±1.15$^{b}$ |
| | 0.12 | 21.66±1.20$^{c}$ | 48.33±0.88$^{c}$ | 63.33±0.88$^{b}$ |
| | 0.17 | 26.67±0.33$^{b}$ | 60.00±0.57$^{b}$ | 80.00±0.88$^{a}$ |
| *Z. officinal, B. nigra* and *P.dodecandra* | 0.07 | 15.00±0.57$^{c}$ | 31.66±1.33$^{c}$ | 55.00±0.57$^{b}$ |
| | 0.12 | 23.33±0.88$^{c}$ | 48.33±1.33$^{c}$ | 65.00±1.73$^{b}$ |
| | 0.17 | 26.66±0.33$^{b}$ | 56.66±0.66$^{b}$ | 80.00±0.33$^{a}$ |
| *A. sativum, Z. officinal* and *N. tobacum* | 0.07 | 15.00±0.57$^{c}$ | 33.33±0.88$^{c}$ | 65.00±2.00$^{b}$ |
| | 0.12 | 26.66±0.08$^{b}$ | 56.66±0.33$^{b}$ | 73.33±0.66$^{b}$ |
| | 0.17 | 26.66±0.33$^{b}$ | 58.33±0.33$^{b}$ | 85.00±1.00$^{a}$ |
| *A. sativum, B. nigra and N. tobacum* | 0.07 | 13.33±0.33$^{c}$ | 26.66±1.20$^{c}$ | 48.33±0.88$^{c}$ |
| | 0.12 | 23.333±0.88$^{c}$ | 50.00±1.00$^{b}$ | 61.66±1.20$^{b}$ |
| | 0.17 | 25.33±0.88$^{b}$ | 56.66±0.33$^{b}$ | 78.33±0.66$^{a}$ |
| *B. nigra, P. dodecandra* and *N. tobacum* | 0.07 | 13.33±0.33$^{c}$ | 30.00±0.00$^{c}$ | 56.66±0.33$^{b}$ |
| | 0.12 | 20.00±0.57$^{c}$ | 46.67±1.20$^{c}$ | 65.00±1.00$^{b}$ |
| | 0.17 | 28.33±0.33$^{b}$ | 58.33±0.33$^{b}$ | 83.33±0.88$^{a}$ |
| *A. sativum, P. dodecandra* and *N. tobacum* | 0.07 | 16.66±0.88$^{c}$ | 33.33±0.66$^{c}$ | 58.33±1.20$^{b}$ |
| | 0.12 | 26.33±0.66$^{b}$ | 58.33±0.33$^{b}$ | 75.00±0.57$^{a}$ |
| | 0.17 | 28.66±0.33$^{b}$ | 65.00±0.57$^{b}$ | 88.33±0.88$^{a}$ |
| *Z. officinal, B. nigra* and *N. tobacum* | 0.07 | 13.33±0.33$^{c}$ | 28.33±0.33$^{c}$ | 50.00±0.57$^{b}$ |
| | 0.12 | 21.66±0.66$^{c}$ | 46.66±1.20$^{c}$ | 55.00±2.08$^{b}$ |
| | 0.17 | 26.66±0.88$^{b}$ | 60.00±0.57$^{b}$ | 80.00±1.15$^{a}$ |
| *Z. officinal, P. dodecandra* and *N.tobacum* | 0.07 | 20.00±0.57$^{c}$ | 40.00±0.00$^{c}$ | 78.33±0.66$^{a}$ |
| | 0.12 | 26.67±0.33$^{b}$ | 58.33±0.66$^{b}$ | 78.33±0.33$^{a}$ |
| | 0.17 | 31.66±0.33$^{b}$ | 58.33±0.66$^{b}$ | 88.66±0.88$^{a}$ |
| Five combined botanical | 0.07 | 15.00±0.57$^{c}$ | 28.33±0.33$^{c}$ | 53.33±0.66$^{b}$ |
| | 0.12 | 25.00±0.57$^{b}$ | 55.00±0.00$^{b}$ | 71.66±0.88$^{b}$ |
| | 0.17 | 25.00±0.57$^{b}$ | 55.00±0.57$^{b}$ | 80.00±0.57$^{a}$ |
| Deltamethrin dust | 0.07 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| | 0.12 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| | 0.17 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| Control | - | 0.0±0.00$^{c}$ | 0.0±0.00$^{c}$ | 0.0±0.00$^{c}$ |

Means followed by the same letter within column are not significantly different at 5% level,HSD

Ethanol extract of *P. dodecandra* showed a significant ($P< 0.05$) toxicity effect (93.33% mortality) whereas *B. nigra* showed lowest (60%) mortality at 72 hours after treatment application with the concentration of 0.17 %w/w (table 4). The present result showed that *P. dodecandra* is more toxic than the other botanical extracts. It might be due to the nature of bioactive compounds that could be more toxic to *B. pisorum* adults. Studies also indicated that *P. dodecandra* could also possess antifeedant, oviposition repellant, ovicidal, and adulticidal properties against bruchids [16].

Table 4 Mean ± SE percent mortality of *B. pisorum* at different rate of the individual ethanol extracts botanicals at 24, 48, 72 hours exposure time

| Treatment | Dose (w/w) | Mean ± SE adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *Allium sativum* | 0.07 | 11.66±0.33$^{b}$ | 28.33±0.88$^{b}$ | 45.00±1.15$^{c}$ |
| | 0.12 | 18.33±0.88$^{b}$ | 36.66±2.02$^{b}$ | 53.33±1.20$^{b}$ |
| | 0.17 | 23.33±0.66$^{b}$ | 48.33±0.33$^{b}$ | 70.00±1.00$^{b}$ |
| *Zingeber officinal* | 0.07 | 13.33±0.33$^{b}$ | 28.33±0.33$^{b}$ | 51.65±0.33$^{b}$ |
| | 0.12 | 20.00±0.57$^{b}$ | 45.00±1.00$^{b}$ | 58.33±1.45$^{b}$ |
| | 0.17 | 26.66±1.45$^{a}$ | 58.33±0.33$^{a}$ | 81.66±0.33$^{a}$ |
| *Brassica nigra* | 0.07 | 10.00±0.00$^{b}$ | 20.00±0.57$^{c}$ | 36.65±0.33$^{c}$ |
| | 0.12 | 15.00±1.00$^{b}$ | 31.66±0.33$^{b}$ | 40.00±0.57$^{c}$ |
| | 0.17 | 21.66±0.88$^{b}$ | 43.33±2.18$^{b}$ | 60.00±2.08$^{b}$ |
| *Nicotiana tobacum* | 0.07 | 20.00±0.57$^{b}$ | 40.00±1.52$^{b}$ | 60.00±2.30$^{b}$ |
| | 0.12 | 21.66±1.20$^{b}$ | 46.66±1.20$^{b}$ | 66.66±1.76$^{b}$ |
| | 0.17 | 28.33±1.20$^{a}$ | 60.00±0.57$^{a}$ | 88.33±0.33$^{a}$ |
| *Photolacca dodecandra* | 0.07 | 18.33±1.20$^{b}$ | 40.00±2.00$^{b}$ | 60.00±0.57$^{b}$ |
| | 0.12 | 25.00±1.15$^{a}$ | 51.66±1.45$^{a}$ | 73.33±1.20$^{b}$ |
| | 0.17 | 35.00±0.57$^{a}$ | 71.66±0.66$^{a}$ | 93.33±0.00$^{a}$ |

Means followed by the same letter within column are not significantly different at 5% level, HSD

The ethanol extracts of *P. dodecandra* and *N.tobacum* combinations with the concentration of 0.17%w/w caused the highest mortality (88.33%) at 72hr exposure time.However, *A. sativum* and *B. nigra* powder combinations at the rate 0.17%w/w showed the least toxicity effect (63.33%) against the target insect (table 5). The high percent mortality caused by the presence of *P. dodecandra* could be the presence of saponin and other secondary metabolites in the plant [17].

Other studies also reported that the reason might be due to the presence of Terpenoids that has been detected in the ethanolic extract of *P. dodecandra* [11, 18]. Many studies reported that bioactivity of locally available botanical extracts needs simple methods with acceptable and cost effective approaches compared to synthetic pesticides.

Table 5 Mean (±SE) percent mortality of the two botanical ethanol extract combinations at different rates at the 24, 48, 72 hours exposure time

| Treatment | Dose (w/w) | Mean± SE adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *A. sativum* and *Z. officinal* | 0.07 | 16.66±0.33$^{b}$ | 35.00±1.00$^{b}$ | 56.66±1.45$^{b}$ |
| | 0.12 | 20.00±1.52$^{b}$ | 40.00±0.57$^{b}$ | 58.33±0.33$^{b}$ |
| | 0.17 | 25.00±0.57$^{a}$ | 53.33±0.66$^{a}$ | 75.00±1.00$^{a}$ |
| *A. sativum* and *B. nigra* | 0.07 | 10.00±0.00$^{b}$ | 23.33±0.60$^{c}$ | 41.66±0.88$^{c}$ |
| | 0.12 | 15.00±0.57$^{b}$ | 31.66±0.33$^{b}$ | 45.00±0.57$^{c}$ |
| | 0.17 | 21.66±0.66$^{b}$ | 45.00±0.57$^{b}$ | 63.33±0.33$^{b}$ |
| *Z. officinal* and *B. nigra* | 0.07 | 16.66±0.33b | 23.33±0.33$^{c}$ | 43.33±0.33$^{c}$ |
| | 0.12 | 15.00±1.00$^{b}$ | 35.00±0.57$^{b}$ | 46.66±0.88$^{c}$ |
| | 0.17 | 23.33±0.88$^{b}$ | 48.33±1.66$^{b}$ | 68.33±1.20$^{b}$ |
| *A. sativum* and *P. dodecandra* | 0.07 | 16.66±0.33$^{b}$ | 33.33±0.88b | 58.33±1.45$^{b}$ |
| | 0.12 | 20.00±1.33$^{b}$ | 43.33±0.88$^{b}$ | 61.65±0.88$^{b}$ |
| | 0.17 | 28.33±0.33a | 58.33±0.66$^{a}$ | 81.66±0.33$^{a}$ |
| *Z. officinal* and *P. dodecandra* | 0.07 | 15.00±0.57b | 33.33±1.20$^{b}$ | 58.33±0.66$^{b}$ |
| | 0.12 | 21.66±0.33$^{b}$ | 45.00±0.57$^{b}$ | 63.33±0.33$^{b}$ |
| | 0.17 | 30.00±0.57a | 60.00±0.57$^{a}$ | 85.00±1.15$^{a}$ |
| *B. nigra* and *P. dodecandra* | 0.07 | 11.66±0.33$^{b}$ | 25.00±0.57$^{b}$ | 65.65±0.33$^{b}$ |
| | 0.12 | 18.33±0.88$^{b}$ | 40.00±1.52$^{b}$ | 55.00±2.08$^{b}$ |
| | 0.17 | 25.00±1.00a | 50.00±1.00$^{a}$ | 71.66±1.66$^{b}$ |
| *A. sativum* and *N. tobacum* | 0.07 | 13.33±0.66b | 28.33±1.20b | 50.00±0.57$^{b}$ |
| | 0.12 | 18.33±0.88$^{b}$ | 40.00±1.52$^{b}$ | 58.33±0.33$^{b}$ |
| | 0.17 | 23.33±0.88$^{b}$ | 51.66±0.88$^{a}$ | 75.00±0.57$^{a}$ |
| *Z. officinal* and *N. tobacum* | 0.07 | 13.33±0.33$^{b}$ | 31.66±0.88$^{b}$ | 55.00±0.57$^{b}$ |
| | 0.12 | 20.00±0.57$^{b}$ | 45.00±0.57$^{b}$ | 61.66±1.20$^{b}$ |
| | 0.17 | 26.66±1.20$^{a}$ | 55.00±0.00$^{a}$ | 80.00±0.57$^{a}$ |
| *B. nigra* and *N. tobacum* | 0.07 | 15.00±0.57$^{b}$ | 30.00±0.57$^{b}$ | 45.00±1.00$^{c}$ |
| | 0.12 | 16.66±0.33$^{b}$ | 36.66±1.45b | 51.65±2.02$^{b}$ |
| | 0.17 | 23.33±0.88$^{b}$ | 48.33±0.88$^{b}$ | 70.00±0.57$^{b}$ |
| *P. dodecandra* and *N. tobacum* | 0.07 | 18.33±0.33$^{b}$ | 38.33±0.66$^{b}$ | 58.33±0.88$^{b}$ |
| | 0.12 | 21.66±0.33$^{b}$ | 46.66±0.88$^{b}$ | 66.65±1.85$^{b}$ |
| | 0.17 | 28.33±1.20$^{b}$ | 60.00±0.57$^{a}$ | 88.33±0.33$^{a}$ |

Means followed by the same letter within column are not significantly different at 5% level,HSD

The combination of three botanicals (*Z. officinal, N. tobacum* and *P. dodecandra)* extracted by ethanol caused the highest percent mortality (83.33%) by the dose of 0.17w/w at 72hr exposure time. But, the lower mortality (70%) was observed by the treatment of *A. sativum, Z. officinal* and *B. nigra* by the same amount and time exposure. The low toxic effect could be due to the constituents from the botanical plant powders were not toxic to the target insect body [19]. Studies also reported that the toxicity effect of plant extracts depends on the active constituents of the plant extract [20].

Table 6 Mean ±SE percent mortality of the three botanical ethanol extract combinations at different rate of concentrations during the exposure time of 24, 48, 72 hours

| Treatment | Dose (w/w) | Mean±SE Adult mortality | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| *A. sativum, Z. officinal* and *B. nigra* | 0.07 | 11.66±0.33$^{c}$ | 26.66±0.88$^{b}$ | 43.33±0.33$^{d}$ |
| | 0.12 | 16.66±0.33$^{c}$ | 35.00±0.57$^{b}$ | 35.00±0.57$^{d}$ |
| | 0.17 | 25.00±1.00$^{b}$ | 50.00±0.57$^{b}$ | 70.00±1.00$^{c}$ |
| *A. sativum, Z. officinal* and | 0.07 | 15.00±0.00$^{c}$ | 30.00±0.57$^{c}$ | 50.00±1.15$^{c}$ |
| *P. dodecandra* | 0.12 | 20.00±0.57$^{c}$ | 41.66±0.33$^{c}$ | 58.33±0.66$^{c}$ |
| | 0.17 | 26.66±0.33$^{b}$ | 55.00±0.00$^{b}$ | 78.33±0.88$^{b}$ |
| *A. sativum, B. nigra* and | 0.07 | 13.33±0.33$^{c}$ | 26.66±0.66$^{c}$ | 50.00±0.57$^{c}$ |
| *P. dodecandra* | 0.12 | 18.33±0.66$^{c}$ | 38.33±1.20$^{c}$ | 53.33±0.66$^{c}$ |
| | 0.17 | 26.66±0.88$^{b}$ | 53.33±0.33$^{b}$ | 73.33±0.88$^{c}$ |
| *Z. officinal, B. nigra* and | 0.07 | 11.66±0.33$^{c}$ | 26.66±0.66$^{c}$ | 48.33±1.33$^{d}$ |
| *P. dodecandra* | 0.12 | 16.33±0.66$^{c}$ | 40.00±1.00$^{c}$ | 53.33±0.88$^{c}$ |
| | 0.17 | 23.33±0.88$^{c}$ | 51.66±0.33$^{b}$ | 75.00±0.57$^{b}$ |
| *A. sativum, Z. officinal* and *N.* | 0.07 | 13.33±0.33$^{c}$ | 28.33±0.66$^{c}$ | 50.00±0.57$^{c}$ |
| *tobacum* | 0.12 | 18.33±0.33$^{c}$ | 40.00±1.00$^{c}$ | 58.33±0.66$^{c}$ |
| | 0.17 | 25.00±0.57$^{c}$ | 53.33±0.88$^{b}$ | 76.66±1.20$^{c}$ |
| *A. sativum, B. nigra* and *N.* | 0.07 | 13.33±0.33$^{c}$ | 25.00±1.00$^{c}$ | 43.33±0.33$^{d}$ |
| *tobacum* | 0.12 | 16.66±0.33$^{c}$ | 36.66±1.20$^{c}$ | 51.66±1.45$^{c}$ |
| | 0.17 | 21.66±0.88$^{c}$ | 50.00±1.15$^{b}$ | 71.66±1.45$^{c}$ |
| *B. nigra, P. dodecandra* and *N.* | 0.07 | 11.66±0.33$^{c}$ | 26.66±0.66$^{c}$ | 48.33±1.33$^{d}$ |
| *tobacum* | 0.12 | 18.33±0.66$^{c}$ | 40.00±0.57$^{c}$ | 55.00±1.00$^{c}$ |
| | 0.17 | 26.66±0.33$^{b}$ | 55.00±0.57$^{b}$ | 76.65±0.33$^{b}$ |
| *A. sativum, P. dodecandra* and *N.* | 0.07 | 15.00±0.57$^{b}$ | 31.66±0.66$^{c}$ | 55.00±1.00$^{c}$ |
| *tobacum* | 0.12 | 20.00±0.57$^{c}$ | 43.33±0.33$^{c}$ | 58.33±0.33$^{c}$ |
| | 0.17 | 26.66±0.88$^{b}$ | 56.65±0.33$^{b}$ | 80.00±0.57$^{b}$ |
| *Z. officinal, B. nigra* and *N. tobacum* | 0.07 | 11.66±0.33$^{c}$ | 26.65±0.33$^{c}$ | 46.00±0.33$^{d}$ |
| | 0.12 | 16.66±0.66$^{c}$ | 36.66±0.33$^{c}$ | 51.66±0.33$^{c}$ |
| | 0.17 | 23.33±0.66$^{c}$ | 37.00±0.57$^{c}$ | 73.33±0.88$^{c}$ |
| | 0.07 | 16.66±0.33$^{c}$ | 38.33±0.88$^{c}$ | 61.65±0.66$^{c}$ |
| *Z. officinal, P. dodecandra* and *N.* | 0.12 | 21.66±0.33$^{c}$ | 51.65±0.66$^{b}$ | 73.33±1.45$^{c}$ |
| *tobacum* | 0.17 | 26.66±1.20$^{b}$ | 60.00±1.15$^{b}$ | 83.33±0.88$^{c}$ |
| Deltamethrin dust | 0.07 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| | 0.12 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| | 0.17 | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ |
| Control | | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{e}$ |

Means followed by the same letter within column are not significantly different at 5% level,HSD

**Dose response toxicity of botanical extracts against *B. pisorum***

The dose response estimate of $LC_{50}$ and $LC_{90}$ with 95% confidence limit for individual botanicals aqueous extracts against adult *B. pisorum* was presented in table 7. The smaller dose for $LC_{50}$, 1.48 (0.86-1.92) and $LC_{90}$ 0.61 (0.11-0.98) values was observed in *P. dodecandra* were 1.48 (0.86-1.92) and 0.61 (0.11-0.98) respectively after 24 hours exposure time. While the extract of *B. nigra* botanicals at 24 hours exposure time showed less insecticidal activity and the $LC_{50}$ and $LC_{90}$ values were 1.93 (0.25-2.53) and 0.99 (0.00-1.55) respectively. No mortality was recorded in the untreated check.4In agreement to the current study [21] reported that water extract botanicals were effective insecticides against adult coleopteran pests in the $LC_{50}$ and $LC_{90}$ on the first day after exposure. The higher dosage plant powders in addition to directly pests poison; it affects the egg laying and larval development of *B. pisorum* [22].

Table 7 Dose response assay of single botanical aqueous extracts against *B. pisorum* under laboratory conditions.

| Treatment | 24 hr | | 48 hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| *A.sativum* | 0.91 (0.10-1.36) | 1.78 (0.95-2.25) | 0.35 (0.02-0.70) | 1.02 (0.33-1.41) | 0.17 (0.00-0.44) | 0.65 (0.09-1.03) |
| *Z. officinal* | 0.68 (0.10-1.07) | 1.61 (0.97-2.13) | 0.26 (0.00-0.58) | 0.88 (0.21-1.27) | 0.13 (0.00-0.39) | 0.57 (0.04-0.96) |
| *B. nigra* | 0.99 (0.00-1.55) | 1.93 (0.25-2.53) | 0.35 (0.00-0.74) | 1.12 (0.27-1.57) | 0.17 (0.00-0.47) | 0.71 (0.07-1.12) |
| *N.tobacum* | 0.64 (0.09-1.03) | 1.55 (0.89-2.03) | 0.22 (0.00-0.53) | 0.79 (0.15-1.17) | 0.07 (0.00-0.31) | 0.42 (0.00-0.83) |
| *P.dodecandra* | 0.61 (0.11-0.98) | 1.48 (0.86-1.92) | 0.14 (0.00-0.44) | 0.64 (0.03-1.06) | 0.05 (0.00-0.26) | 0.34 (0.00-0.76) |

The dose response results showed that the percentage of mortality is directly proportional to the concentration of the powder and exposure time. The synergistic

effect on the $LC_{50}$ and $LC_{90}$ values with low dose effect of the combination of the *P. dodecandra* and *N. tobacum* aqueous extracts were 0.56(0.04-0.96) and 1.48 (0.76-1.98) respectively at 24 hours exposure time (table 8). The result indicated in Table 9 also showed similar observation when tested by the combination of the three botanical aqueous extracts against *B. pisorum*. This study indicated that *P. dodecandra* can protect the stored pea weevils by applying a small dose of the extract.

Table 8 Dose response assay of the two botanicals combinations aqueous extracts and tested against *B. pisorum* under laboratory conditions

| Treatment | 24 hr | | 72hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| *A. sativum* and *Z. officinal* | 0.93 (0.11-1.38) | 1.81 (0.97-2.28) | 0.30 (0.02-0.61) | 0.92 (0.28-1.29) | 0.10 (0.00-0.35 | 0.49 (0.01-0.90)) |
| *A. sativum* and *B. nigra* | 0.97 (0.0-1.53) | 1.90 (0.25-2.48) | 0.35 (0.01-0.74) | 1.11 (0.25-1.56) | 0.17 (0.00-0.47 | 0.70 (0.08-1.10)) |
| *Z. officinal* and *B. nigra* | 0.853 (0.04-1.3) | 1.88 (1.02-2.63) | 0.33 (0.02-0.67) | 1.01 (0.33-1.39) | 0.11 (0.00-0.37) | 0.55 (0.01-0.97) |
| *A. sativum* and *P. dodecandra* | 0.91 (0.29-1.28) | 1.76 (1.22-2.20) | 0.21 (0.00-0.52) | 0.78 (0.12-1.19) | 0.07 (0.00-0.30) | 0.41 (0.00-0.82) |
| *Z. officinal* and *P. dodecandra* | 0.67 (0.11-1.06) | 1.59 (0.95-2.09) | 0.15 (0.0-0.45) | 0.66 (0.02-1.08) | 0.06 (0.0-0.29) | 0.38 (0.0-0.80) |
| *B. nigra* and *P. dodecandra* | 1.00 (0.09-1.48) | 1.96 (1.07-2.54) | 0.35 (0.01-0.70) | 1.06 (0.32-1.47) | 0.14 (0.00-0.42) | 0.61 (0.04-01.02) |
| *A. sativum* and *N. tobacum* | 0.85 (0.11-1.30) | 1.78 (1.02-2.33) | 0.34 (0.02-0.67) | 1.01 (0.34-1.39) | 0.10 (0.00-0.37) | 0.53 (0.01-0.94) |
| *Z. officinal* and *N. tobacum* | 1.742 (0.98-2.24) | 0.844 (0.12-1.28) | 0.96 (0.29-1.34) | 0.31 (0.01-0.63) | 0.47 (0.0-0.87) | 0.09 (0.00-0.34) |
| *B. nigra* and *N. tobacum* | 0.88 (0.12-1.33) | 1.83 (1.07-2.39) | 0.33 (0.02-0.67) | 1.01 (0.34-1.39) | 0.15 (0.00-0.43) | 0.64 (0.05-1.04) |
| *P.dodecandra* and *N. tobacum* | 0.56 (0.04-0.96) | 1.48 (0.76-1.98) | 0.18 (0.00-0.48) | 0.71 (0.07-1.12) | 0.06 (0.00-0.28) | 0.37 (0.0-0.78) |

Table 9 Dose response assay of the combination of the three botanical aqueous extracts against *B. pisorum* in 24, 48 and 72 hours exposure under laboratory conditions

| Treatment | 24 hr | | 48 hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| *A.sativum*, *Z. officinal* and *B. nigra* | 0.99 (0.09-1.46) | 1.93 (1.06-2.50) | 0.35 (0.01-0.71) | 1.07 (0.30-1.48) | 0.16 (0.00-0.45) | 0.66 (0.06-1.06) |
| *A. sativum*, *Z. officinal* and *P. dodecandra* | 0.83 (0.13-1.26) | 1.70 (0.96-2.16) | 0.30 (0.01-0.63) | 0.94 (0.27-1.32) | 0.08 (0.00-0.33) | 0.46 (0.00-0.87) |
| *A. sativum*, *B. nigra* and *P. dodecandra* | 0.96 (0.30-1.35) | 1.87 (1.32-2.40) | 0.28 (0.01-(0.61) | 0.93 (0.25-1.32) | 0.095 (0.00-0.35) | 0.497 (0.00-0.92) |
| *Z. officinal*, *B. nigra* and *P. dodecandra* | 0.85 (0.11-1.30 | 1.78 (1.02-2.33) | 0.30 (0.01-0.63) | 0.96 (0.26-1.36) | 0.09 (0.00-0.35) | 0.50 (0.00-0.92) |
| *A. sativum*, *Z. officinal* and *N. tobacum* | 0.81 (0.09-1.25) | 1.71 (0.92-2.21) | 0.28 (0.01-0.60) | 0.90 (0.23-1.290) | 0.05 (0.00-0.28) | 0.37 (0.00-0.79) |
| *A.sativum*, *B. nigra* and *N. tobacum* | 0.972 (0.09-1.44) | 1.89 (1.03-2.43) | 0.34 (0.01-0.69) | 1.04 (0.31-1.44) | 0.13 (0.00-0.41) | 0.60 (0.03-1.01) |
| *B. nigra*, *P. dodecandra and N. tobacum* | 1.00 (0.09-1.48) | 1.96 (1.07-2.54) | 0.33 (0.02-0.67) | 1.01 (0.33-1.39) | 0.09 (0.00-0.34) | 0.48 (0.00-0.90) |
| *A.sativum*, *P. dodecandra* and *N. tobacum* | 0.62 (0.01-1.08) | 1.56 (0.58-2.10) | 0.30 (0.01-0.61) | 0.91 (0.28-1.28) | 0.09 (0.00-0.33) | 0.46 (0.00-0.87) |
| *Z. officinal*, *B. nigra* and *N. tobacum* | 0.99 (0.09-1.46) | 1.93 (1.06-2.50) | 0.34 (0.01-0.68) | 1.03 (0.34-1.42) | 0.12 (0.00-0.39) | 0.58 (0.02-1.00) |
| *Z. officinal*, *P. dodecandra* and *N. tobacum* | 0.68 (0.12-1.06) | 1.58 (0.96-2.06) | 0.18 (0.00-0.49) | 0.72 (0.05-1.14) | 0.06 (0.00-0.29) | 0.39 (0.00-0.81) |

The dose response estimate of $LC_{50}$ and $LC_{90}$ with 95% confidence limit for individual ethanolic extract botanical against adult *B. pisorum* is presented in table 10. The smaller dose for $LC_{50}$, 0.58 (0.02-1.02) and $LC_{90}$ 1.48 (0.58-1.99) values was observed in *P. dodecandra* after 24 hours exposure time. While the extract of *B. nigra* botanicals at 24 hours exposure time showed less insecticidal activity with the $LC_{50}$ and $LC_{90}$ values of 1.06

(0.00-1.65) and 2.08 (0.08-2.93) respectively. No mortality was recorded in the untreated check. The *P. dodecandra* dose response showed that it has potent insecticidal activity toward pea weevils. This might be due the presence of bioactive compounds exerting toxicity effect against *B. pisorum*.

Table 10 Dose response assay of the individual botanical extracted by ethanol and treated against *B. pisorum* under laboratory conditions

| Treatment | 24 hr | | 48 hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | LC50 | LC90 | LC50 | LC90 | LC50 | LC90 |
| *A.sativum* | 1.02 (0.00-1.59) | 2.01 (0.17-2.71) | 0.46 (0.05-0.81) | 1.22 (0.57-1.61) | 0.15 (0.00-0.44) | 0.65 (0.03-1.07) |
| *Z. officinal* | 1.01 (0.08-1.50) | 1.98 (1.09-2.58) | 0.344 (0.01-0.68) | 1.038 (0.33-1.43) | 0.117 (0.00-0.38) | 0.558 (0.01-0.97 |
| *B. nigra* | 1.06 (0.00-1.65) | 2.08 (0.08-2.93 | 0.70 (0.21-1.05) | 1.50 (0.97-1.87) | 0.22 (0.00-0.55) | 0.85 (0.13-1.27) |
| *N. tobacum* | 0.68 (0.09-1.09) | 1.68 (1.01-2.29) | 0.18 (0.00-0.49) | 0.75 (0.05-1.17) | 0.08 (0.00-0.32) | 0.44 (0.00-0.86) |
| *P. dodecandra* | 0.58 (0.02-1.02) | 1.48 (0.58-1.99) | 0.21 (0.00-0.51) | 0.77 (0.13-1.17) | 0.09 (0.00-0.33) | 0.47 (0.01-0.87) |

The dose response assay of the two and the three botanicals extracted by ethanol treated as combinations against *B. pisorum* under laboratory conditions is indicated in table 11. The smaller dose for $LC_{50}$, 0.74 (0.00-1.34) and $LC_{90}$ 1.82 (0.00-2.71) values was observed in *A. sativum* and *N. tobacum* combination and the $LC_{50}$ of 0.96 (0.30-1.35), $LC_{90}$ of 1.87 (1.32-2.40) values for *Z.officinal, N. tobacum and P.dodecandra* (table 12) 24 hours exposure time. While the extract of *A. sativum* and *B. nigra* botanicals combinations at 24 hours exposure time showed less insecticidal activity with the LC50 and LC90 values of 1.08 (0.00-1.68) and 2.12 (0.04-2.99) respectively. Whereas the less insecticidal activity was observed in the dose response assay LC 50 and LC90 of *Z.official, B.nigra and N.tobacum* with the values of 1.06 (0.0-1.65) and 2.08 (0.08-2.93) respectively. The results indicated that the two botanical combinations showed more toxic than the three combinations in the dose response assy. This might be due to the interaction effect of the botanicals during their combinations. The dose response result showed that ethanolic extract botanicals of single and their combinations observed higher toxicity than the aqueous extracts. This might be due to the bioactive

components like terpionoids which have toxicity effect against the adult pea weevil. The dose response the tested botanical extracts in this present study varied with plant species and period of exposure. Some scholars suggested that botanical pesticides could have direct or delayed insecticidal effects [ 24]. The delayed effect operates indirectly by inhibiting reproduction and development (oviposition, larval penetration into the seed and adult emergence) that can be evaluated after a complete cycle of development of the pest.

Table 11 Dose response assay of the combination of the two botanicals ethanolic extracts against *B. pisorum* under laboratory conditions

| Treatment | 24 hr | | 48 hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| *A. sativum* and *Z. officinal* | 0.97 (0.28-1.37) | 1.91 (1.34-2.51) | 0.23 (0.00-0.57) | 0.88 (0.13-1.31) | 0.07 (0.00-0.32) | 0.38 (0.00-0.80) |
| *A. sativum* and *B. nigra* | 1.08 (0.00-1.68) | 2.12 (0.04-2.99) | 0.53 (0.08-0.89) | 1.33 (0.71-1.72) | 0.16 (0.00-0.47) | 0.72 (0.04-1.15) |
| *Z. officinal* and *B. nigra* | 0.86 (0.01-1.39) | 1.86 (0.70-2.60) | 0.53 (0.09-0.88) | 1.29 (0.70-1.67) | 0.16 (0.00-0.46) | 0.69 (0.04-1.12) |
| *A. sativum* and *P. dodecandra* | 0.95 (0.30-1.34) | 1.86 (1.30-2.36) | 0.28 (0.01-0.61) | 0.94 (0.24-1.34) | 0.07 (0.00-0.32) | 0.45 (0.00-0.88) |
| *Z. officinal* and *P. dodecandra* | 0.90 (0.16-1.33) | 1.80 (1.08-2.31) | 0.36 (0.00-0.61) | 0.94 (0.25-1.33) | 0.08 (0.00-0.32) | 0.46 (0.00-0.88) |
| *B. nigra* and *P. dodecandra* | 1.00 (0.00-1.57) | 1.97 (0.22-2.61) | 0.34 (0.00-0.72) | 1.10 (0.26-1.54) | 0.14 (0.00-0.42) | 0.62 (0.02-1.04) |
| *A. sativum* and *N. tobacum* | 0.74 (0.00-1.34) | 1.82 (0.00-2.71) | 0.33 (0.01-0.69) | 1.05 (0.30-1.47) | 0.12 (0.0-0.39) | 0.57 (0.01-0.99) |
| *Z. officinal* and *N. tobacum* | 1.01 (0.08-1.50) | 1.98 (01.09-2.58) | 0.29 (0.01-0.63) | 0.97 (0.25-1.37) | 0.09 (0.00-0.35) | 0.49 (0.0-0.92) |
| *B. nigra* and *N. tobacum* | 0.95 (0.10-1.43) | 2.01 (1.22-2.85) | 0.29 (0.00-0.65) | 1.03 (0.20-1.47) | 0.15 (0.00-0.44) | 0.65 (0.03-1.07) |
| *P.dodecandra* and *N. tobacum* | 0.816 (0.19-1.21) | 1.77 (1.17-2.33) | 0.20 (0.00-0.52) | 0.79 (0.09-1.21) | 0.08 (0.00-0.33) | 0.46 (0.00-0.88) |

Table 12 Dose response assay of the three botanicals ethanolic extracts treated as combinations against *B. pisorum* under laboratory conditions

| Treatment | 24 hr | | 48 hr | | 72 hr | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| *A. sativum*, *Z. officinal* and *B. nigra* | 1.04 (0.0-1.62) | 2.04 (0.15-2.77) | 0.53 (0.10-0.88) | 1.29 (0.71-1.66) | 0.16 (0.00-0.46) | 0.71 (0.04-1.15) |
| *A. sativum*, *Z. officinal* and *P. dodecandra* | 1.02 (0.22-1.46) | 1.99 (1.33-2.60) | 0.30 (0.01-.65) | 1.01 (0.24-1.42) | 0.12 (0.00-0.39) | 0.57 (0.02-0.99) |
| *A.sativum*, *B. nigra* and *P. dodecandra* | 1.03 (0.08-1.53) | 2.02 (1.11-2.68) | 0.34 (0.01-0.71) | 1.08 (0.30-1.51) | 0.11 (0.00-0.38) | 0.56 (0.01-0.99) |
| *Z. officinal*, *B. nigra* and *P. dodecandra* | 1.02 (0.00-1.59) | 2.01 (0.17-2.71) | 0.34 (0.01-0.707) | 1.08 (0.29-1.50) | 0.13 (0.00-0.41) | 0.60 (0.02-1.02) |
| *A. sativum*, *Z. officinal* and *N. tobacum* | 1.05 (0.07-1.54) | 2.04 (1.13-2.74) | 0.33 (0.01-0.69) | 1.05 (0.31-1.47) | 0.12 (0.00-0.39) | 0.57 (0.02-0.99) |
| *A. sativum*, *B. nigra* and *N. tobacum* | 1.13 (0.03-1.66) | 2.23 (1.30-3.44) | 0.45 (0.05-0.80) | 1.19 (0.55-1.58) | 0.17 (0.00-0.46) | 0.69 (0.06-1.11) |
| *B. nigra*, *P. dodecandra* and *N. tobacum* | 0.99 (0.00-1.55) | 1.93 (0.25-2.53) | 0.34 (0.01-0.71) | 1.07 (0.31-1.50) | 0.13 (0.00-0.41) | 0.60 (0.02-1.02) |
| *A. sativum*, *P. dodecandra* and *N. tobacum* | 0.91 (0.13-1.36) | 1.87 (1.12-2.46) | 0.30 (0.01-0.63) | 0.98 (0.26-1.38) | 0.09 (0.00-0.35) | 0.49 (0.00-0.92) |
| *Z. officinal*, *B. nigra* and *N. tobacum* | 1.06 (0.0-1.65) | 2.08 (0.08-2.93) | 0.34 (0.01-0.71) | 1.09 (0.28-1.53) | 0.14 (0.00-0.42) | 0.63 (0.03-1.05) |
| *Z. officinal*, *P. dodecandra* and *N. tobacum* | 0.964 (0.30-1.35) | 1.87 (1.32-2.40) | 0.20 (0.0-0.52) | 0.78 (0.09-1.19) | 0.06 (0.00-0.30) | 0.41 (0.00-0.83) |

## Effect of botanical extracts on the weight loss of field pea grains

During this evaluation water extracted *P. dodecandra* and *N. tobacum* combination gave the lowest percent of weight loss (0.35%) of the grain. Field pea grains treated by the combination of *Z. officinal*, *P. dodecandra* and *N. tobacum* at highest dose (0.17w/w) resulted in the lowest percent of weight loss (0.64%) (Table13). The botanical *P. dodecandra* aqueous extracts resulted in the lowest percent of weight loss of the grain. Moreover, ethanol extract of *P. dodecandra* indicated the minimum level weight loss (0.25%) of the grain after treatment application (table14). Similar studies reported that *P. dodecandra* extracts exerted no weight loss record in maize weevils [11].

Table 13 Effect of single botanical aqueous extracts on the weight loss of field pea grains

| Treatments | Mean ±SE 0.07w/w | Mean ±SE 0.12w/w | Mean ±SE 0.17w/w |
|---|---|---|---|
| *A. sativum* | 2.29±0.22$^{b}$ | 1.82±0.17$^{a}$ | 1.30±0.13$^{a}$ |
| *Z. officinal* | 2.08±0.40$^{b}$ | 1.54±0.45$^{a}$ | 1.29±0.32$^{a}$ |
| *B. nigra* | 4.20±0.07$^{c}$ | 3.34±0.35$^{c}$ | 3.11±0.23$^{c}$ |
| *N. tobacum* | 1.40±0.07$^{a}$ | 1.05±0.16$^{a}$ | 0.71±0.17$^{ab}$ |
| *P. dodecandra* | 1.32±0.13$^{a}$ | 0.41±0.09$^{ab}$ | 0.00±0.00$^{abc}$ |
| Treatments with two botanical combinations | | | |
| *A. sativum* and *Z. officinal* | 1.89±0.54$^{a}$ | 1.68±0.27$^{a}$ | 1.03±0.45$^{a}$ |
| *A. sativum and B. nigra* | 2.94±0.42$^{b}$ | 2.55±0.30$^{b}$ | 2.19±0.31$^{b}$ |
| *Z. officinal* and *B. nigra* | 3.06±0.17$^{c}$ | 2.46±0.38$^{b}$ | 2.15±0.24$^{b}$ |
| *A. sativum* and *P. dodecandra* | 1.82±0.02$^{a}$ | 1.11±0.13$^{a}$ | 0.64±0.06$^{ab}$ |
| *Z. officinal* and *P. dodecandra* | 1.64±0.16$^{a}$ | 0.96±0.28$^{ab}$ | 0.62±0.14$^{ab}$ |
| *B. nigra* and *P. dodecandra* | 2.75±0.03$^{b}$ | 2.20±0.44$^{b}$ | 1.55±0.12$^{a}$ |
| *A. sativum* and *N. tobacum* | 1.95±0.15$^{a}$ | 1.42±0.15$^{a}$ | 1.00±0.14$^{a}$ |
| *Z. officinal* and *N. tobacum* | 1.80±0.22$^{a}$ | 1.54±0.11$^{a}$ | 1.00±0.18$^{a}$ |
| *B. nigra* and *N. tobacum* | 2.83±0.07$^{b}$ | 2.21±0.27$^{b}$ | 1.89±0.20$^{a}$ |
| *P. dodecandra* and *N. tobacum* | 1.40±0.01$^{a}$ | 0.72±0.13$^{ab}$ | 0.35±0.08$^{ab}$ |
| Treatments with three botanicals combinations | | | |
| *A. sativum, Z. officinal* and *B. nigra* | 2.86±0.15$^{b}$ | 2.22±0.29$^{b}$ | 1.55±0.30$^{a}$ |
| *A. sativum, Z. officinal* and *P. dodecandra* | 1.90±0.13$^{a}$ | 1.24±0.22$^{a}$ | 0.84±0.14$^{ab}$ |
| *A. sativum, B. nigra* and *P. dodecandra* | 2.61±0.13$^{b}$ | 1.86±0.21$^{a}$ | 1.44±0.12$^{a}$ |
| *Z. officinal, B. nigra* and *P. dodecandra* | 2.51±0.12$^{b}$ | 1.58±0.18$^{a}$ | 1.45±0.16$^{a}$ |
| *A. sativum, Z. officinal* and *N. tobacum* | 1.88±0.18$^{a}$ | 1.49±0.18$^{a}$ | 1.07±0.15$^{a}$ |
| *A. sativum, B. nigra* and *N. tobacum* | 2.48±0.09$^{b}$ | 2.05±0.22$^{b}$ | 1.69±0.18$^{a}$ |
| *B. nigra, P. dodecandra* and *N. tobacum* | 2.52±0.17$^{b}$ | 1.83±0.03$^{a}$ | 1.25±0.12$^{a}$ |
| *A. sativum, P. dodecandra* and *N. tobacum* | 1.73±0.06$^{a}$ | 1.130.15$^{a}$ | 0.65±0.10$^{ab}$ |
| *Z. officinal, B. nigra* and *N. tobacum* | 2.52±0.12$^{b}$ | 1.97±0.30$^{a}$ | 1.68±0.20$^{a}$ |
| *Z. officinal, P. dodecandra* and *N. tobacum* | 1.63±0.12$^{b}$ | 0.99±0.20$^{ab}$ | 0.64±0.11$^{ab}$ |
| Deltamethrin dust | 0.00±0.00$^{abc}$ | 0.00±0.00$^{abc}$ | 0.00±0.00$^{abc}$ |
| Control(untreated) | 4.50±0.26$^{c}$ | 4.50±0.26$^{c}$ | 4.50±0.26$^{c}$ |

Means followed by same letter (level case) within column are not significantly different P<0.05%

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Table 14 Effect of botanicals extracted by ethanolic extracts on the weight loss of field pea grains

| Treatment | Mean ± SE 0.07w/w | Mean ± SE 0.12w/w | Mean ± SE 0.17w/w |
|---|---|---|---|
| *A.sativum* | 2.92±0.15$^{b}$ | 2.27±0.22$^{b}$ | 1.74±0.09$^{a}$ |
| *Z. officinal* | 2.01±0.77$^{b}$ | 2.08±0.40$^{b}$ | 1.66±0.36$^{a}$ |
| *B. nigra* | 4.26±0.07$^{c}$ | 3.90±0.32$^{c}$ | 3.32±0.56$^{c}$ |
| *N. tobacum* | 2.27±0.23$^{b}$ | 1.57±0.14$^{a}$ | 1.13±0.23$^{a}$ |
| *P. dodecandra* | 1.75±0.09$^{a}$ | 0.97±0.10$^{ab}$ | 0.25±0.15$^{ab}$ |
| Treatments with two botanical combinations | | | |
| *A. sativum* and *Z. officinal* | 2.75±0.16$^{b}$ | 2.23±0.15$^{b}$ | 1.74±0.14$^{a}$ |
| *A. sativum* and *B. nigra* | 3.79±0.08$^{c}$ | 3.25±0.07$^{c}$ | 2.58±0.27$^{b}$ |
| *Z. officinal* and *B. nigra* | 3.61±0.21$^{c}$ | 3.11±0.26$^{c}$ | 2.49±0.45$^{b}$ |
| *A. sativum* and *P. dodecandra* | 2.36±0.08$^{b}$ | 1.53±0.17$^{a}$ | 1.04±0.07$^{a}$ |
| *Z. officinal* and *P. dodecandra* | 2.23±0.21$^{b}$ | 1.38±0.29$^{a}$ | 0.95±0.26$^{ab}$ |
| *B. nigra* and *P. dodecandra* | 3.25±0.11$^{c}$ | 2.41±0.17$^{b}$ | 1.79±0.36$^{a}$ |
| *A. sativum* and *N. tobacum* | 2.60±0.12$^{b}$ | 1.95±0.08$^{a}$ | 1.48±0.09$^{a}$ |
| *Z. officinal* and *N. tobacum* | 2.42±0.33$^{b}$ | 1.83±0.27$^{a}$ | 1.39±0.29$^{a}$ |
| *B. nigra* and *N. tobacum* | 3.47±0.08$^{c}$ | 2.85±0.14$^{b}$ | 2.23±0.39$^{b}$ |
| *P. dodecandra* and *N. tobacum* | 2.05±0.09$^{b}$ | 1.14±0.18$^{a}$ | 0.69±0.19$^{ab}$ |
| Treatments three botanical combinations | | | |
| *A. sativum, Z. officinal and B. nigra* | 3.35±0.09$^{c}$ | 2.79±0.11$^{b}$ | 2.23±0.29$^{b}$ |
| *A. sativum, Z.officinal* and *P. dodecandra* | 2.41±0.10$^{b}$ | 1.69±0.17$^{a}$ | 1.22±0.15$^{a}$ |
| *A. sativum, B. nigra* and *P.dodecandra* | 3.01±0.11$^{c}$ | 2.47±0.17$^{b}$ | 1.78±0.22$^{a}$ |
| *Z. officinal, B. nigra* and *P. dodecandra* | 2.95±0.16$^{b}$ | 2.43±0.21$^{b}$ | 1.72±0.38$^{a}$ |
| *A. sativum, Z. officinal* and *N. tobacum* | 2.56±0.18$^{b}$ | 1.98±0.15$^{a}$ | 1.51±0.17$^{a}$ |
| *A. sativum, B. nigra* and *N. tobacum* | 3.23±0.03$^{c}$ | 2.66±0.09$^{b}$ | 2.10±0.26$^{b}$ |
| *B. nigra, P. dodecandra* and *N. tobacum* | 2.89±0.06$^{b}$ | 2.08±0.16$^{b}$ | 1.52±0.30$^{a}$ |
| *A.sativum,P.dodecandra* and *N. tobacum* | 2.89±0.06$^{b}$ | 2.08±0.16$^{b}$ | 1.52±0.30$^{a}$ |
| *Z. officinal, B. nigra* and *N. tobacum* | 3.09±0.23$^{c}$ | 2.52±0.18$^{b}$ | 2.01±0.38$^{b}$ |
| *Z. officinal, P. dodecandra* and *N. tobacum* | 2.13±0.26$^{b}$ | 1.33±0.30$^{a}$ | 0.98±0.26$^{ab}$ |
| Deltamethrin dust | 0.00±0.00$^{d}$ | 0.00±0.00$^{abc}$ | 0.00±0.00$^{abc}$ |
| Control (Untreated) | 4.50±0.26$^{c}$ | 4.51±0.26$^{c}$ | 4.51±0.26$^{c}$ |

Means followed by same letter within column are not significantly different P<0.05%

**Effect of Botanicals on Germination of grain seeds**

Results of mean percent germination of pea grains treated by botanicals in aqueous and ethanolic extracts are given in table 15 and 16 respectively. Results from germination bioassay showed that the botanical extracts did not show such a negative effect on the germination ability of the seed in all concentration. Pea grains treated with all botanicals were almost (greater than 95%) germinated. The highest rate of germination (98.66%) was recorded on the pea grain treated by *P. dodecandra* water and ethanol extracts at the rate of 0.17% w/w. The pea grains treated with botanicals extracted by the two solvents (water and ethanol) have no more variation in terms of extractive solvents and dose rates used. Researchers also reported that plant extract treatments used for the control of bruchid pest on cowpea did not exhibit significant or adverse effect on the germination rate of the grain [24].

Table 15 Mean percentage grain germination of pea grains treated by botanicals extracted by water

| Treatment | Mean ±SE 0.07w/w | Mean ±SE 0.12w/w | Mean ±SE 0.17w/w |
|---|---|---|---|
| *A. sativum* | 94.66±0.66[b] | 96.00±1.15[a] | 96.66±0.66[a] |
| *Z. officinal* | 94.66±0.66[b] | 96.66±0.66[a] | 97.33±0.66[a] |
| *B. nigra* | 93.33±0.66[b] | 94.66±0.66[b] | 96.00±0.00[a] |
| *N. tobacum* | 94.66±0.66[b] | 96.66±0.66[a] | 97.33±0.66[a] |
| *P. dodecandra* | 96.00±1.15[a] | 98.00±0.00[a] | 98.66±0.66[a] |
| Treatment of two botanical combinations | | | |
| *A. sativum* and *Z. officinal* | 94.66±0.66[b] | 96.00±1.15[a] | 96.66±0.66[a] |
| *A. sativum* and *B. nigra* | 93.33±0.66[b] | 95.33±0.66[b] | 96.00±0.00[a] |
| *Z. officinal* and *B. nigra* | 94.00±0.00[b] | 95.33±0.66[b] | 96.00±0.00[a] |
| *A. sativum* and *P. dodecandra* | 94.66±0.66[b] | 96.66±0.66[a] | 97.33±0.66[a] |
| *Z. officinal* and *P.dodecandra* | 94.66±0.66[b] | 96.66±0.66[a] | 97.33±0.66[a] |
| *B. nigra* and *P. dodecandra* | 94.00±1.15[b] | 96.00±0.00[a] | 96.66±0.66[a] |
| *A. sativum* and *N. tobacum* | 94.66±0.66[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *Z. officinal* and *N. tobacum* | 94.66±0.66[b] | 96.00±0.00[a] | 97.33±0.66[a] |
| *B. nigra* and *N. tobacum* | 93.33±0.66[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *P. dodecandra* and *N. tobacum* | 95.33±0.66[b] | 97.33±0.66[a] | 98.00±1.15[a] |
| Treatment of three botanical combinations | | | |
| *A. sativum, Z. officinal* and *B. nigra* | 93.33±0.66[b] | 95.33±0.66[b] | 96.00±0.00[a] |
| *A. sativum, Z. officinal* and *P. dodecandra* | 94.66±0.66[b] | 94.66±1.76[b] | 97.33±0.66[a] |
| *A. sativum, B. nigra* and *P. dodecandra* | 93.33±0.66[b] | 95.33±0.66[b] | 96.00±0.00[a] |
| *Z. officinal, B. nigra* and *P. dodecandra* | 94.00±1.15[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *A. sativum, Z. officinal* and *N. tobacum* | 93.33±0.66[b] | 96.00±1.15[a] | 96.66±0.66[a] |
| *A. sativum, B. nigra* and *N. tobacum* | 93.33±0.66[b] | 95.33±0.66[b] | 96.00±0.00[a] |
| *B. nigra, P. dodecandra* and *N. tobacum* | 94.00±1.15[b] | 96.00±0.00[a] | 97.33±0.66[a] |
| *A. sativum, P. dodecandra* and *N. tobacum* | 94.66±0.66[b] | 96.66±0.66[a] | 97.33±0.66[a] |
| *Z. officinal, B. nigra* and *N. tobacum* | 93.33±0.66[b] | 93.33±0.66[b] | 96.66±0.66[a] |
| *Z. officinal, P. dodecandra* and *N. tobacum* | 95.33±0.66[b] | 96.00±1.15[a] | 97.33±0.66[a] |
| Deltamethrin dust | 99.33±0.66[a] | 99.33±0.66[a] | 100±0.00[a] |
| Control (untreated) | 92.66±0.66[c] | 92.66 ±0.66[c] | 92.66±0.66[c] |

Mean followed by same letter within column are not significantly different (P<0.05%)

Table 16 Mean percentage grain germination of pea grains treated with botanicals extracted by ethanol

| Treatment | Mean ±SE 0.07w/w | Mean ±SE 0.12w/w | Mean ±SE 0.17w/w |
|---|---|---|---|
| *A. sativum* | 94.66±0.66[b] | 95.33±0.66[b] | 96.65±0.66[a] |
| *Z. officinal* | 93.33±0.66[b] | 95.33±0.66[b] | 96.66±0.65[a] |
| *B. nigra* | 92.66±0.66[c] | 93.32±0.66[b] | 94.00±0.00[b] |
| *N. tobacum* | 94.66±0.66[b] | 96.00±1.15[a] | 97.33±0.66[a] |
| *P. dodecandra* | 94.65±0.66[b] | 96.66±0.66[a] | 98.66±0.66[a] |
| Treatment of two botanical combinations | | | |
| *A. sativum* and *Z. officinal* | 92.66±0.66[c] | 95.33±0.66[b] | 96.00±0.00[a] |
| *A. sativum* and *B. nigra* | 92.66±0.66[c] | 93.33±0.66[b] | 94.65±0.66[b] |
| *Z. officinal* and *B. nigra* | 92.66±0.66[c] | 94.00±0.00[b] | 95.33±0.66[b] |
| *A. sativum* and *P. dodecandra* | 93.33±0.66[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *Z. officinal* and *P. dodecandra* | 93.33±0.66[b] | 95.33±0.66[b] | 97.33±0.66[a] |
| *B. nigra* and *P. dodecandra* | 93.33±0.66[b] | 94.00±0.00[b] | 95.33±0.66[b] |
| *A. sativum* and *N. tobacum* | 93.32±0.66[b] | 94.65±0.66[b] | 95.33±0.66[b] |
| *Z. officinal* and *N. tobacum* | 93.33±0.66[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *B. nigra* and *N. tobacum* | 93.33±0.66[b] | 94.00±0.66[b] | 95.33±0.66[b] |
| *P. dodecandra* and *N. tobacum* | 94.66±0.66[b] | 95.33±0.66[b] | 97.33±0.65[a] |
| Treatment of three botanical combinations | | | |
| *A. sativum, Z. officinal* and *B. nigra* | 92.66±0.66[c] | 93.33±0.66[b] | 95.33±0.66[b] |
| *A. sativum, Z. officinal* and *P. dodecandra* | 93.33±0.66[b] | 95.33±0.66[b] | 96.66±0.66a |
| *A. sativum, B. nigra* and *P. dodecandra* | 93.33±0.66[b] | 94.66±0.66[b] | 95.33±0.66[b] |
| *Z. officinal, B. nigra* and *P. dodecandra* | 93.33±0.66[b] | 94.66±0.65[b] | 96.66±0.67[a] |
| *A. sativum, Z. officinal and N. tobacum* | 92.66±0.66[c] | 94.66±0.65[b] | 96.65±0.66[a] |
| *A. sativum, B. nigra* and *N. tobacum* | 92.66±0.66[c] | 94.66±0.65[b] | 96.00±0.00[a] |
| *B. nigra, P. dodecandra* and *N. tobacum* | 93.33±0.66[b] | 95.33±0.65[b] | 96.66±0.66[a] |
| *Allium sativum, P. dodecandra* and *N. tobacum* | 94.00±0.00[b] | 95.33±0.66[b] | 96.66±0.66[a] |
| *Z. officinal, B. nigra* and *N. tobacum* | 92.66±0.66[c] | 94.66±0.66[b] | 96.00±0.66[a] |
| *Z. officinal, P. dodecandra* and *N. tobacum* | 94.67±0.66[b] | 96.66±0.66[b] | 97.33±0.66[a] |
| Deltamethrin dust | 99.33±0.66[a] | 99.33±0.66[a] | 100±0.00[a] |
| Control (untreated) | 92.66±0.66[c] | 92.66 ±0.66[c] | 92.66±0.66[c] |

Means followed by same letter within column are not significantly different P<0.05%

## Conclusion

From the present study it is concluded that botanical extract could be used as an alternative to synthetic insecticides. In other words, botanical pesticides can be used to minimize the side effects of chemical insecticides. Botanical extracts have different insecticidal chemical properties and modes of action such as repellents, antifeedants, toxicity, growth inhibition on insects. Among the botanical insecticides tested in this study *P. dodecandra* could be preferable to use for the control of *B. pisorum.* Moreover, water extracts of the mixture of *Z. officinal, P. dodecandra* and *N.tobacum* showed better insecticidal properties against *B. pisorum.*

## Recommendations

Based on the present study, it is recommended to use tested botanical insecticides particularly *P. dodecandra* for the control of *B. pisorum.* Further investigations on the target plant extracts are under large scale storage conditions and its long term toxicity effect on mammals and beneficial organisms is recommended.

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**Original Article: Open access**

# Experimental and Numerical Evaluation of Rensile Strength of Horse Hair-Glass Fiber/Epoxy Hybrid Composites

Mamaru Wutabachew[1*], Fekadu Berihun[2]

[1]Department of Mechanical and industrial Engineering, Debre Markos institute of technology, Debre Markos, Ethiopia.

[2]Department of Mechanical and industrial Engineering, Debre Markos institute of technology, Debre Markos, Ethiopia.

***Corresponding author:** mamwuta@gmail.com

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## Abstract

Natural fibers are environmentally friendly with less weight and energy conservation than the synthetic fibers. The present study paper assesses the mechanical properties of the hybrid composite which is composed of horsehair and glass fiber materials using experimental methods and Digimat simulation. The impact of weight percentage and orientation of fiber on mechanical properties such as tensile strength is investigated. The total weight of the fiber was 70% and the matrix was 30%.The specimen was prepared using the hand layup process. The weight percentage increment was 20% and the orientation of the fiber was $0^0$, $90^0$ and $0\text{-}90^0$. By doing the Digimat simulation and testing the composite; it was rich that there is a basic effect of the weight percentage and the orientation in mechanical property of composite. With different weight percentage of fiber, the maximum tensile strength was 282.97 Mpa in the experiment result and 290 Mpa in the Digimat simulation result which is 56% of glass fiber plus 14% of horse hair fiber plus 30% of epoxy resin. Whereas in the orientation of the fiber $0^0$ has the maximum tensile strength which is 127.2 Mpa in the experiment result and 150.12Mpa in the Digimat simulation result of the presents study. The test outcomes were drawn and the conclusion was made with the comparison of the result of horsehair/epoxy, glass fiber

/epoxy and hybrid composite with software simulation. Further investigation is recommended to test with other animal hairs.

***Keywords;*** Tensile strength; Horse hair-glass; fibre composites; epoxy composites

## Introduction

The availability and distribution of horses and its hair in Ethiopia within the region have been written clearly. Ethiopia is endowed with abundant agricultural resources. The statics showed that there are more than 2 million horse's lives and it is about 33.5% of the African and 3.45% of the world horse population [1]. The population of the horse in Ethiopia ranked the first in Africa and 8$^{th}$ in the world. According to livestock census of Ethiopia, the distribution of horses in the regions indicated that the Oromia region ranked the first and highest population of the horse. Around 1,176301 horses which are 58% of the total population in the country is found in the Oromia region. The second-ranked region in horse population was the South nation and nationality which accounts 451,799 (22.27%) of the horse population in the country. The third one was the Amhara region, which is 396231 (19%) of the horse population in the country. The Tigray region accounts 0.73% of the horse population in the country [2]. The horsehair is not sold in some regions, except the Amhara region. Commonly, the horse hair is bought from the markets of Amhara region particularly Engibara town in Awi zone.

Due to increasing the need for composite material, the development of natural fiber composite is a new topic in recent research and technology. By increasing environmental awareness, many researchers shift their interest in research of in natural fiber composite materials. Natural fibers are a capability of replacing synthetic materials and their related products. It has less weight and energy conservation applications compared with synthetic fibers. Moreover, when compared to synthetic, natural fibers have low cost, low weight, abundant and renewable resources [3]. Due to this reason, the present study has come up with a natural resource that is available in our environment. The horse hair is naturally gifted fiber and there is no extraction process. The present study focused on horsehair and glass fiber with epoxy resin hybrid composite materials with mechanical properties to replace conventional material. Moreover, in this study the mechanical properties such as the tensile strength of glass fiber and horsehair reinforced polymer hybrid composite

material with different orientation and weight percentage of the fiber is evaluated. [3]

## Materials and Methods

### Description of the study

For the present study, the specimen preparation was conducted in Dejen Aviation Industry which is located at the city Bishfotu in Oromia region. After preparing the specimen the experiment was conducted in Defense Engineering College in mechanical design laboratory room of the same city. Materials for the current study included commercially available epoxy resin that has been used as a matrix and the reinforcement materials, commercially available raw material horsehair/glass from the local market.

### Description of the study materials

#### *Horsehair*

The horse hair for the study was bought from the market of Amhara regional state, Engibara town in Awl zone. The horsehair fiber was collected from the live horse. The specific strength and stiffness of horsehair was compared to those of copper wire with the same diameter [4].

#### *E- glass fiber*

E–Glass fiber is one of the most important artificial class of reinforcement material specially used in polymer composites. Glass fiber has a low thermal coefficient, low dielectric coefficient, and high electrical resistance. This property depends on additives and curing agents [5]. It is obtained from Dejen Aviation Industry which is located at the city Bishfotu in Oromia region.

#### *Hardener*

The hardener is used as a binder during the production. Araldite HY951 hardener was used in this study. It has low viscosity, cure at room temperature, good mechanical strength, and good resistance to atmospheric and chemical degradation. The epoxy resin is obtained from Kadisco Paint Factory Addis Ababa

#### *Remover*

Wax was used to safely remove the prepared specimen from the mold. The remover bought from market.

#### *Epoxy resin*

One of the properties of epoxy resin has good additive properties. The additive property along with its high mechanical strength, low shrinkage, chemically resistant high diffusion density, low viscous and better electric insulation capacity was accepted for the experiment. It is easily reinforced with natural (horsehair) and E glass fibers. LY-501 type of epoxy was used in the current study [6]. The epoxy resin was obtained from Kadisco Paint Factory Addis Ababa lafto sub city around wuha limat.

**Procedures for preparation of laminated experimental specimens**

Each composite laminate was prepared from the mixture of Epoxy, horse hair fiber, and E-glass fibers. The horsehair used in this procedure was untreated and free from chemicals. Then an open mold of aluminum plate with a dimension of 300X300 mm was prepared and the prepared composite was cut for each test. Using the rule of mixtures [7] the various fiber weight proportions were calculated to achieve laminates with 0:100, 100:0, 80:20, 20:80, 60:40 and 40:60 ratios with former being the ratio of fiber and the latter is horse hair. The horse hair and E-Glass fibers with the total weight of the composite fiber contain 70%. The composite material had six layers and the orientation of fiber was $[0^0]$, $[90^0]$, $[0^0/90^0]$. Laminates were composed of plates of different layer materials or layers of fiber-reinforced lamina prepared with the same matrix material. After the above process, horse hair and glass fiber were cut based on the length of the mold. Based on the calculation of weight proportion appropriate amount of horse hair fibers, glass fiber and epoxy resin were considered. The first layer of the specimen was placed in the mold based on the calculated amount of fiber. In the Next step epoxy resin LY501 and the hardener HY-951 were mixed with a ratio of 2:1 before preparing the first layered fibers and applying a mixture of epoxy resin and hardener on the first layer fiber. Then unidirectional E- glass fiber had prepared into required mold size and positioned over other fibers. Again, the calculated amount of epoxy resin and hardener mixture was applied over E-glass fiber. The second layer was prepared with horse hair a fiber was placed over a prepared E glass fiber and again a mixture of epoxy resin and hardener was applied. The resin mixture was spread by hand layup method uniformly around the corners. After spreading resin mixture Deadweight was applied over the open mold to remove air. **A**fter some time, the laminate was removed from the open mold and put in suitable temperature for curing. Finally, the composite was dried for seven days and the specimens were prepared by cutting the plat using a grinder with the required dimension of each test [8].

**Tensile Test**

According to American Society of Teeth Manufacturing (ASTM–D 3039) standard tensile tests on composite specimens were the young modulus of elasticity of glass fiber. And the horsehair hybrid reinforced polymer was done to observe the behavior of hybrid

material under load. The test specimen in tensile testing or tension testing was a fundamental material science test in which a test specimen is subjected to uniaxial tension until specimen failure. The results of the test were commonly used to select a material for quality control and application.

The result of the present test is also used to predict how a material will react under other types of forces. The material properties such as ultimate tensile strength, maximum elongation and reduction in the area are measured in a tensile test. The properties like Young's modulus and yield strength also determined from these measurements (figure 1).

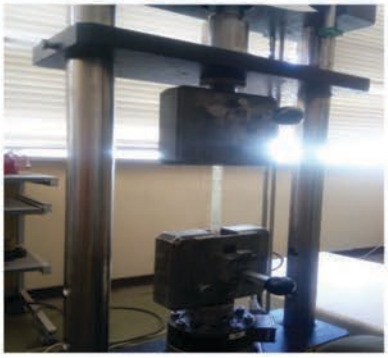

Figure 1 A universal testing machine for tensile test (Photo taken by Mamaru wutabachew, 2019)

**Specimen size**

The most commonly used specimens for American society of teeth manufacturing (ASTM 3039) were constant rectangular cross-section 25mm (1 in) wide, 250 mm (10 in) long and 4mm thickness [9,10]. Optionally, tabs were used to bonded the ends of the specimen to prevent gripping damage (figure 2). For each test, a composite of three specimens were tested and the average value of each test was taken for analysis.

Figure 2 Hybrid spacemen sample for tensile test (Photo taken by Mamaru wutabachew, 2019)

**Data analysis**

Both experimental and software results of the composite materials were analyzed and plotted.

**For the experiment analysis**: three sample spacemen were taken for each weight percentage and orientation. During the test, the force-elongation value and the stress-strain result as well as graph, were generated. The stress-strain graph plotted was due to the change in the area of the specimen after the test. In this case, there was a change in area due to changes in length and thickness of specimen. To find each stress it needs a measurement of each specimen accurately. But, for the present study, all the experimental graphs in tensile strength were plotted from the data of force versus deformation. For plotting stress-strain curves we have two stress cases such as true stress and engineering stress.

In the present study the load and elongation of the three sample specimens were added and divided by three to get the average value of three specimen load and elongation. The stress was found from the average load per unit original area and the strain was found from the average deformation per unit original length of specimen. Then the resulting stress-strain graph was plotted in the tensile test of this work. The maximum tensile strength of each sample taken and the result of the average value were calculated and the modules of elasticity of the material in each test were the slope of the stress-strain curve.

**In case of software simulation**: For predicting the tensile strength, Digmat Software was used. This software is developed for composite material. This is a semi-analytical mean field homogenization module or the same as the analytical calculation but, the difference is that in the stress-strain graph the result of stress in each strain value known. In analytical only, the calculated value is known but, in this software, the result of each stress is known with corresponding strain. The mean-field homogenization (MF) module of the software was used. In the material model fiber type, elasticity and isotropy of the material were selected. In the microstructure phase, the phase-type and phase behavior were filled. Next to this, the number of layers, the thickness of each layer and the orientation of the layer were filled.

The loading type was also selected. Then the result was plotted. The stress-strain diagram of the software result was liner, because the material property that was selected in the software was liner, elastic and isotropic. The software results were also the same as to the

experimental result. The main difference between them was only some deviation of the error in software results.

**Result and Dissection**

**Validation of software and experimental result**

The figures 3 and 4 showed the stress-strain diagram of hybrid composite with different weight percentage and hybrid composite with different orientation of fiber respectively. As shown in each figure, there was a deviation of result in experiment and software, but the variation of strain was nearly the same. In the graph of the tensile strength was linearly increased thoughout the strain. This might be due to the material property fill-in the software was a liner, elastic and isotropic property. But in the experiment, the graph was not linear. The reason could be because of lots of losses like during specimen preparation accuracy, temperature variation, testing machine adjustment of the material property. Due to these reasons the graph was sometimes linear other times, non-linear. The tensile strength of experimental and software results approaches each other when the load approaches maximum. This study was in line with the studies reported in mechanical properties of glass fiber and natural fiber reinforced polymer composite to increase engineering and technology application [11]. The effect of fiber length and orientation of fiber on the mechanical property of the part was studied and horse hairs have higher tensile strength than glass fiber. The present study also agreed to other studies that horse hair was the best reinforced material used with comparing other fibers [12]. The mechanical properties of glass fiber were investigated and resulted in higher mechanical properties of the material. The study was also conducted by the experiment and digmat simulation to compare the results. Studies also reported that, depending on weight percentage and loading condition of fiber, some natural fiber has higher mechanical properties than glass fiber [13].

**A Hybrid composite with a different weight percentage**

Fig. 3 shows the stress-strain diagram of glass fiber and horsehair fiber with epoxy resin hybrid composite. The result showed four different weight percentages of each fiber but it has the same orientation of fiber. Starting from the initial point to some amount of strain, the stress-strain graph was far apart, but the load reached the maximum tensile stress of the materials in both experimental and software results. Both experimental and software results were also approached.

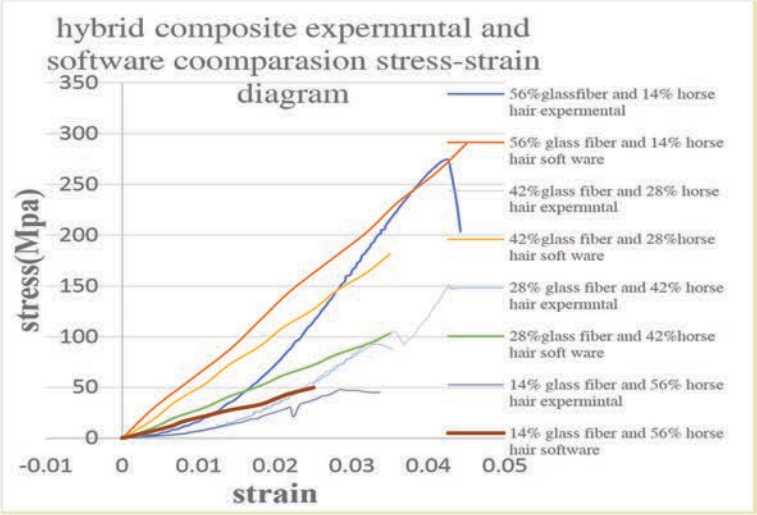


Figure 3 the experimental and software result of hybrid composite with a weight percentage

**B hybrid composite with Orientation**

Figure 4 shows the stress-strain diagram of glass fiber and horsehair with epoxy resin hybrid composite with different orientation of the fiber. The weight percentage of the fibers was equal. The result also showed the maximum tensile strength of experimental result approaches to the software result

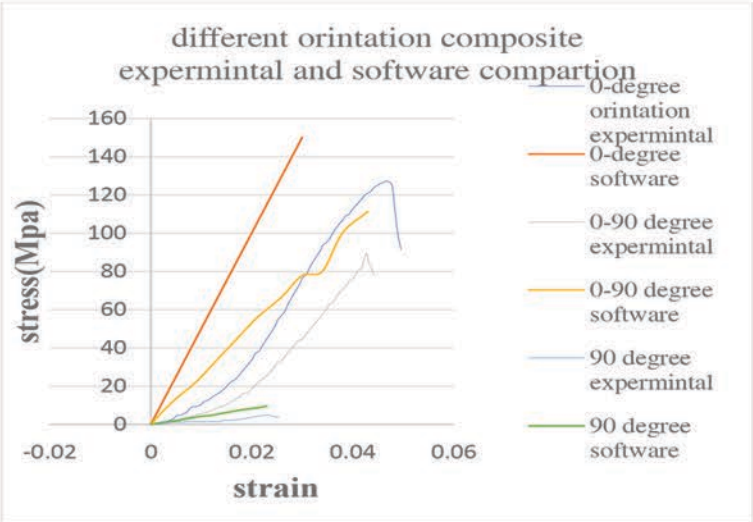


Figure 4 experimental and software result of composite with orientation

Different composite tensile strengths with experimental and software results were also shown similar results (Table 1). The table is showing the maximum tensile strength of 7 different composite materials.

Table1 Shows 7 different composite tensile strengths with experimental and software results

| NO. | Tensile strength (Mpa) | experimental | software | error |
|---|---|---|---|---|
| 1 | 0-degree hybrid | 127.2 | 150.12 | 0.1527 |
| 2 | 90-degree hybrid | 4.833 | 9.565 | 0.2020 |
| 3 | 0-90-degree hybrid | 89.5 | 111.33 | 0.1960 |
| 4 | 14% glass hybrids | 48.87 | 49.758 | 0.0178 |
| 5 | 28% glass hybrids | 93.7 | 102.95 | 0.0898 |
| 6 | 42% glass hybrids | 151.733 | 181.41 | 0.1636 |
| 7 | 56%glass hybrids | 282.97 | 290 | 0.0173 |

The deviation or error of the software and experimental result is calculated by the software result minus experimental result divided by software result.

**Conclusion**

The investigation of glass fiber and horsehair fiber hybrid composite leads to the following conclusions. For tensile test 70% glass fiber/epoxy composite has the average tensile strength was 220.8Mpa but, the hybridization of 56% glass fiber and 14%horse hair the tensile strength was 282.97Mpa which was enhancing the tensile strength approximately 21.97%. In the directions of fiber orientation, $0^0$ has maximum tensile strength but for other direction the tensile strength was reduced. In

hybridization of horse hair and glass fiber, increasing weight proportion of glass fiber and decreasing weight proportion of horse hair fiber was enhancing the tensile strength of the composite.

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# Assessment of some physicochemical parameters and levels of some heavy metals in muscles of Nile Tilapia Fish (*Oreochromis niloticus*) in Bahiregiorgis Lake, Ethiopia

Minbale Admas Teshager[1*] and Derese Yigzaw[1]

[1]Department of Chemistry, College of Natural and Computational Science,
Debre Markos University, P.O. 269, Debre Markos, Ethiopia

[*] **Corresponding author**: (E-mail: minbalead@gmail.com)

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**Abstract**

Today, pollution of aquatic and terrestrial ecosystems with toxic heavy metals are an environmental problem of public health concern. To assess the impact of pollution's physicochemical parameters, heavy metal concentrations in Fishes tissue and water samples of Lake Bahire Giorgies were analyzed. The physicochemical properties were found to be Temperature of 18.6°c, pH 8.2, Electrical conductivity of 82.7 µS/cm, turbidity 348.6 NTU. From these parameters' turbidity of water has been above the standard limits. The concentration of heavy metals were found in order, Fe (0.665 mg/L) > Zn (0.53 mg/L) > Cu(0.185 mg/L) but Cd and Pb did not detect in both samples in edible tilapia fish Fe (34.5 mg/kg) > Zn (31.41 mg/kg) > Cu (9 mg/kg). Except Fe, the concentrations of all metals detected were below the recommended limit from WHO (2008) EEPA and FAO in both samples. In conclusion, the results revealed that heavy metals are more accumulated in fish muscle than in water samples due to bioaccumulation in nature.

**Keywords:** Lake Bahire Giorgies; Fish muscle; Heavy metals

## Introduction

Heavy metals contamination of aquatic ecosystems is the pressing worldwide problem, because of their toxicity, persistence, and ability of bioaccumulation. As compared to other types of aquatic pollution, heavy metals' pollution was less visible but its effects on the ecosystem and human beings have been intensive and very extensive [1-3]. Once they entered the water system, it may precipitate, adsorbed on to the solid surfaces, remain soluble, suspended in water or may be taken up by fauna and eventually accumulate in aquatic organisms, water, sediments and subsequently transferred to human being through the food chain, thus it causes many health problems such as cancer, neuropsychological problems, kidney problems, and other numerous health problems [4]. Therefore, the heavy metals concentrations in aquatic ecosystems were usually determined in water, sediments, and fish tissue [5].

The Lake receives various kinds of pollutants influenced by human activities such as poor agricultural practice, deforestation and natural phenomena inter in the lake through the only Inflow River which is called Shegeze River. It carries runoff from the surroundings catchments approximately 12 km far from the lake and then lay and form higher sediments in the lake which contains heavy metals. These different sources cause the accumulation of heavy metals in the Lake water, suspended solid, sediment, fish tissue, and aquatic plants which affect the growth of fish and are harmful to the consumer [6]. Despite, there were relative works in Ethiopia and African countries the result of the level of concentration of heavy metals reported was different because of different topography and human activities [7].

As far as the researchers' knowledge, there was no previous work reported and documented on Lake Bahire Giorgies East Gojjam Zone, Ethiopia. Therefore, this study was conducted to analysis some physicochemical parameters and comparative study of some selective heavy metals' concentration level of (Cu, Pb, Fe, Cd and Zn) in both lake water and edible fish Nile tilapia (*Oreochromis niloticus*) local name known as ``Kereso`` by using FAAS analysis techniques. Thus, therefore, this study was mainly focused on assessment of some physicochemical parameters of lake water (temperature, pH, Electrical conductivity, turbidity) and comparative study of some heavy metal concentration (Cu, Pb, Fe, Cd and Zn) in lake water and the edible muscles of Nile Tilapia fish species which are the most common types of fish consumed by the local community.

## Materials and methods

### Equipments

Water temperature was measured in-situ, using a Thermometer by dipping in water for about 2 minutes and has recorded in degree Celsius. Electrical conductivity was determined Conductivity meter (Jenway, 3310). The pH of the water was measured using portable digital pH meter by immersing into the lake water at a depth of about 30 cm for 10 seconds. Turbidity has measured by portable turbid meter (Aqua Fluor model 8000-010, Hach, USA). A digital analytical balance (Model ESJ210-4, CHINA) with a precision of ± 0.0001 g was used to weigh the sample, Flame atomic absorption spectroscopy (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and air-acetylene flame atomizer was used for the determination of the selected metals ( Fe, Cu, Zn, Pb, and Cd) in fishes edible muscles and water samples. A drying oven (G.P.O. BOX58, Ambala cantt-133 011, INDIA) was used to dry the sample. The 250 ml round bottom flasks fitted with reflux condenser were used to digest the dried and powdered sample. Filtration funnels and filter paper (Whatt mann – 541) were used for filtration of sample solution after digestion during for sample preparation processes. Volumetric flasks (50,100 and 250 ml) were used during dilution, preservation of samples and preparation of metals standard solutions. A refrigerator (CXFG1685W ESKISEHIR, TURKEY) was used for sample preservation after digestion and before AAS analysis. Micropipettes of size 50-200 µl and 100-1000 µl (Du37516 and YE6K770265, CHINA) were used for measuring reagents used during sample preparation, preparation of standard solutions and spike [9].

### Chemicals and Reagents

Analytical grade chemicals, reagents, distilled and deionized water were used throughout the laboratory work Standard solutions for FAAS (1000mg/l for Cu, Cd, Fe, Pb and Zn nitrate salts). (70%, Spectrosol Nitric acid, (37%) Hydrochloric acid and (35%, Riedel-de Haen) hydrogen peroxide were used in this study.

### Description of sampling sites

Lake Bahire Giorgies is located in North west Ethiopia, Amhara Regional State of East Gojjam administrative zone, in Goncha Siso Enessie district, at the particular reference of Getese-Mani village. The sampling sit is 175 km far from East Gojjam Zone administrative zone town, Debre Markos and 12 km Northeast far from Goncha Siso Enessie districta town, Gendewoyine. It is a highland type lake and is volcanic in its origin. The lake is encircled by hilly and intensive cultivated area to all direction and bounded by three villages (Ybucher-ywya, Eneseikole, and Getesemani-

wafa). The total surface area of the lake is about 86.66 $km^2$ having 5 to 30 m depths and its catchment is about 25 $km^2$ [10]. Geographically, the area is located with coordinates of between $11^0$ 27' 36 '' North of Latitude and $39^0$ 12'56'' East of Longitude. The lake has 2012 meter average altitude above sea levels, environmental average temperature 19.5°c, water temperature is 18.6°c and annual rainfall is 1800 mm [10]

**Sample collection**

Samples were collected from Lake Bahire Giorgies. For physicochemical analysis, three samples were collected per site per sampling date in the field and the water sampler at different depth intervals and homogenized before being sub- sampled.

Table 1 The global position system (GPS) reading during sampling

| Sample stations | Latitude | Longitude | Elevation in (m) |
|---|---|---|---|
| $S_1$ | $11^0$ 27 '37" | $39^0$12' 58'' | 2014 |
| $S_2$ | 11 $^0$47 '40" | 39 $^0$28' 47'' | 2011 |
| $S_3$ | 11 $^0$53 '52" | 39 $^0$43' 45'' | 2012 |

**Water sample collection and transportation**

Water samples were collected from the three selected sites of the Lake in a polyethylene bottle with 1L capacity from the three different sites in a clean, white pre-marked 500 ml polythene container from (10-30 cm depth) triplicate and Composite samples were collected. All sampling bottles were clean before use with detergents and rinsed with deionized water for the sampling purpose transport to Deber Markose University chemistry laboratory for further treatment.

**Fish sample collection and transportation**

The most known and commonly used fish types by the local communities were Nile tilapia (*Oreochromis niloticus*), which were collected based on the availability of fishes at different sites of the lake. (Three per site) were randomly collected with the help of local fishermen within the sampling stations by using plastic nets and the samples were kept in an icebox during transportation to prevent deterioration until analysis for drying and further treatment. Then, the fish were transported into laboratory for further analysis (Figure 1).

Figure1Tilapia fishes sample collection and transportation

**Digestion procedures**

**Wet Digestion Method**

Wet digestion method is the best digestion method that involves the use of both heat and mineral acid/s. Mixtures of strong acids such as HCl and $HNO_3$ can be used to enhance the reaction speed and to ensure complete digestion. It would be carried out in open vessels, in tubes, on a hot plate, in an aluminum heating block or in closed vessels at elevated pressure. This method was chosen based on its less contamination, lower rate of evaporation, cost and time and extraction efficiency so the researcher used wet digestion methods because of this reason [11].

**Digestion procedure of water samples**

Digestion of water takes place after composite the triplicate samples were prepared from the three sampling sites of the Lake water. According to the Methods developed by the United States, Environmental Protection Agency (USEPA) 3005 a 50 ml aliquot of well-mixed water samples were digested in conical flasks covered with a watch glass by adding 1 ml of concentrated (70%, Spectrosol $HNO_3$ and 2.5 ml of concentrated (30%) HCI and heated on a hot plate at 90°C boiled until the solution reached up to the mark (20 ml). Then the beaker was removed and cooled. Each of the digested water samples was filtered through what man filter paper No. 542 into a 100 ml volumetric flask and filled up to the mark with deionized water by the addition of 2 ml of nitric acid to get a clear solution and avoid precipitation of metals. Vigorous boiling has avoided preventing loss of the HCl- $H_2O_2$ azeotrope. Finally, the levels of heavy metals were finally determined using the FAAS instrument.

**Digestion procedure of fish samples**

In order to obtain a representative sample, composites should be prepared by taking the edible tissues from the fish samples at each sampling site. Fish muscles were dried in an electric oven at 100-105°C until constant weight was obtained. The dried samples were crushed using a clean mortar and pestle to produce powdered forms. A homogenized 1 g of each grounded fish, powder samples were weighted using an analytical balance and then transferred into a digestion flask into volume of 250 ml capacity. A mixture of Nitric acid (70%, Spectrosol) and hydrogen peroxide (35%, Riedel-de Haen) in a 1:1 (v/ v) ratio with 20 ml volume were prepared. The prepared samples were added to the mixtures and digested for 4 hour until a clear solution was obtained and the volume reduced to 3-4ml. After that, it was allowed to be cooled and then filtered through Whitman filter paper No.542. Then the filtrate obtained would be diluted to 50 ml in a volumetric flask with deionized water. Finally, the levels of heavy metals in

fish samples were determined using the FAAS instrument.

**Instrument calibration and method detection limit**

The calibration curves were drawn for each of the studied elements from five standard solutions. The correlation coefficients of all the calibration curves were > 0.999 which showed that there is good correlation (relationship) between concentration and absorbance. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank (S blank) by three (LOD = 3 x S blank) .Stock solutions were prepared from analytical grade salts of each metal. Each salt of the metals was weighed and transferred into 250 ml volumetric flasks. The stocks used to prepare the intermediate standards and working standards .The intermediate standards and working standards are prepared from the stock solution by serial dilution with deionized water. After the working standards are prepared the instrument was calibrated to obtain good correlation between absorbance and concentration which is used to determine the unknown concentration of the sample [12 13].

Table 2 Working standard concentration, correlation coefficient and equation of the calibration curves for determination of metals using FAAS

| Metals | Concentration of standard series (ppm) | Correlation values (r) | Regression equation (A*=mc+b) |
|---|---|---|---|
| pb | 0.25,0.5,1,2 | 0.999 | Y=0.001x-0.000 |
| Zn | 0.25,0.5,1.2 | 0.991 | Y=0.187x+0.003 |
| Cu | 0.25,0.5,1,2 | 0.998 | Y=0.040x-0.003 |
| Cd | 0.25,0.5,1,2 | 0.996 | Y=0.001x-0.000 |
| Fe | 0.5,1,2,4 | 0.997 | Y=0.004x+0.000 |

The correlation coefficients of all the calibration curves were > 0.99 and these correlation coefficients indicate very good correlation (positive correlation) between concentration of heavy metals and their absorbance.

**Method Detection Limit**

Method detection limit is the minimum concentration of the analyte which can be detected at 99% confidence level that can be distinguished from statistical fluctuations in a blank, which usually correspond to the standard deviation of blank absorbance times a constant.Usually, it is defined as the amount of analyte that gives a signal equal to three times the standard deviation on the blank. In this study method detection limit of each metal was estimated by digesting three analytical blanks for both lake water and edible fish muscles. Each blank solution was also determined with

FAAS at the same time and condition as with the samples. The method detection limit would be calculated by multiplying the standard deviation of the blank by three [13].

MDL = 3×s, where s = Standard deviation of the blank …….. (1)

The validity of the method was assessed by spiking samples with standards of known concentrations and calculating percentage recoveries. The percentage recoveries for the metals were within the acceptable range of 80 to 120% expected for the elements indicating good accuracy of the analytical procedure [14]. The percentage recovery was calculated by using the following formula.

$$\% \text{ Recovery} = \left[ \frac{C(spiked) - C(non-spiked}{C(added)} \right] \times 100\%$$

---------- (2)

Where: C (spiked) is metal content of the spiked sample

C (non-spiked) metal content of non-spiked sample

C (added) is metal content of known concentration added.

Table 3 Recovery values for the mean concentration of fish sample analyzed by AAS

| Metals | Un-spiked | added | Spiked | Percent recovery |
|---|---|---|---|---|
| Zn | 31.4 ±0.03 | 0.15 | 31.56±0.1 | 94±0.03 |
| Cu | 9± 0.012 | 0.15 | 9.13±0.05 | 87±0.21 |
| Fe | 34.5± 0.01 | 0.15 | 34.64±0.001 | 96±0.15 |

Concentrations in spiked and un-spiked sample are calculated by mean ± SD of the triplicate readings of triplicate sample. The recovery percentages of spiked fish and water sample were obtained as shown in (Table 5&6) and the results for metals under investigation (Cu, Fe and Zn) varied between 86% to 96%.The obtained results are in acceptable range which is mostly no less than 80% and no greater than 120% which revealed that the digestion method and the AAS analysis were reliable.

**Statistical analysis**

The data derived from various determinations were subjected to statistical analysis including mean, Pearson Correlation, and One-way ANOVA (Analysis of Variance) was performed for statistically significant difference in the mean value of heavy metal concentrations and physicochemical parameters between the three sampling sites. Difference in mean values were accepted as being statistically significant if $P < 0.05$. Pearson correlation was used to relate the levels of heavy metals in water, physico-chemical and edible fish muscles. Graphs were also used to compare the level of concentration between samples and within standard limits. All statistical analyses were performed using the SPSS statistical software Version 23 (SPSS Inc, 2016) an Excel spreadsheet, 2007.

## Results and discussion

### Results

### Physicochemical Analysis of Lake Water

Table 4 Value of some physico-chemical parameter of Lake Beharie Georgies water

| Parameters | unit | Physico-chemical Results in sampling sit (mean +SE), n =3 | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | | $S_1$, mean ±SD | $S_2$, mean ±SD | $S_3$,mean ±SD | Mean value | WHO Standard | |
| To | 0c | 18.4±.25 | 19.5±0.62 | 18±0.38 | 18.6±0.42 | 15-30 | 0.120 |
| EC | Us/cm | 75.2±0.05 | 87.6±0.07 | 82.4±0.02 | 82.7±0.05 | 1500 | 0.100 |
| pH | - | 8.4±0.23 | 8.1±0.01 | 8.13±0.03 | 8.2±0.09 | 6.5-9.5 | 0.170 |
| Turb | TUN | 340.3±0.23 | 355.1±0.18 | 350.5±0.24 | 348.6±0.22 | <7 | 0.200 |

### Heavy metals concentration in Lake Bahrie Giorgies water

Table 5 Mean concentration (mg/L) of heavy metals in lake water in dry season.

| Sampling site | Concentration of metals (Mean±SE) n=3 | | | | |
|---|---|---|---|---|---|
| | Pb | Cd | Cu | Zn | Fe |
| $S_1$ | ND | ND | 0.187±0.005 | 0.563±0.058 | 0.667±0.021 |
| $S_2$ | ND | ND | 0.183±0.006 | 0.520±0.004 | 0.660±0.32 |
| $S_3$ | ND | ND | 0.186±0.007 | 0.515±0.004 | 0.667±0.625 |
| Average | | | 0.185±0.006 | 0.532±0.037 | 0.665±0.037 |
| P-value | - | - | 0.744 | 0.234 | 0.973 |
| WHO2004 | 0.01 | 0.003 | 2 | 3 | 0.3 |
| EEPA 2003 | 0.5 | 1 | 2 | 5 | 1 |

ND=Not detected

### Accumulation of metals in edible Tilapia fish muscles

Table 6 Mean concentration (mg/kg) of heavy metals in Tilapia Fish muscle

| Sampling site | Concentration of metals (Mean±SE) mg/kg n=3 | | | | |
|---|---|---|---|---|---|
| | Cd | Pb | Cu | Zn | Fe |
| S1 | ND | ND | 8.1±0.025 | 31.2±0.0004 | 34.9±0.021 |
| S2 | ND | ND | 8.82±0.025 | 31.53±0.011 | 34.2± 0.032 |
| S3 | ND | ND | 10.01±0.038 | 31.5±0.004 | 34.75±0.067 |
| Av. mean value | - | - | 9±0.029 | 31.41±0.005 | 34.5±0.028 |
| P-value | - | - | 0.087 | 0.200 | 0.750 |
| WHO 2008 | 0.05 | 0.5 | 20 | 40 | 30 |
| FOA 2008 | 2 | 1.5 | 30 | 40 | 30 |

### Discussion Physicochemical Analysis

### Water temperature

Temperature is one of the important factors affecting aquatic environments for two reasons. First, water temperature affects

nearly all other water parameters and second aquatic organisms are adapted to a certain temperature range. It exerts an important effect on metal speciation because most chemical reaction rates are highly sensitive to temperature change [14]. Due to increased temperature may affect both uptake and elimination rates of metals, so net bioaccumulation may or may not increase. The three sampling sites were not significantly varied (P >0.05) in temperature between the three sampling sites. That means the temperature was the same throughout the sampling sites. The minimum means the value of temperature was recorded at the sample three sites 18 ± 0.38°c while the relative maximum value was observed at the sample two sites 19.53°c and the mean value was 18.6°c the value was within the standard limits of WHO (2008) (Table 4) and have not negative effect for surviving of fishing.

**pH**

Since, pH depends on the carbon dioxide and carbonate-bicarbonate equilibrium is a crucial indication of water quality, an impact on the solubility and bioavailability of metals in natural water. The lower the pH indicates the higher the solubility of heavy metals, and thus increase in metal bioavailability In this study, the pH water was found to be slightly alkaline where pH varied from 8.64 to 8.75 and the mean value 8.2. Hence, these pH values do not support the bioavailability of most dissolved heavy metals. The pH of this study was not significantly varied (P= 0.17) among the three sampling points of the Lake. The pH values of this Lake water were within the range of the WHO limit for aquatic life (Table 4). It has been reported that the pH between these standards was appropriate for increased fish production. A decrease in pH raises the solubility of metals, for example, weathering and solubility of minerals (limestone or dolomite) in water becomes more rapid if pH is low. However, high pH values tend to precipitate heavy metals as hydroxides [14]. A decrease in pH may also dissolve metals-carbonate complexes, releasing metals ions into the water column. During the sampling period of this study of all the sites, pH of the water measured to be alkaline. It was not expected to find high amounts of dissolved metals in water samples.

**Conductivity of water**

It is a relatively linear function of the concentration of dissolved ions. The more ions those are present in water and wastewater, the higher the conductivity of water. The conductivity levels of Lake Bahire Giorgies ranged from 82.7µS/cm to 87.6µs/cm and the mean value was

82.7μS/cm. The EC values of this Lake were lower than the WHO (2008) standard limit (Table 4) and the lake have low EC value and did not have negative effects on the life of aquatic animals.

**Turbidity**

As shown in Table 4, the mean value of turbidity recorded in lake Bahire Giorgies was 348.6 NTU and ranged to 340.25-355.07 NTU which was higher than the WHO 2008 standards from this result the lake water is not suitable for aquatic animals especially fishery activities in this study area.

Heavy metals concentration and Accumulation in Lake Water and edible Tilapia fish muscles

The result obtained from AAS analysis has shown that these heavy metals such as Fe, Cu and Zn were detected in the fish sample. But the two toxic metals Pb and Cd were not detected in the fish sample.

**Iron (Fe)**

The mean concentration of Fe in sample one were 0.667 mg/l, 0.660 mg/l in sample two and 0.667 mg/l in sample three. The average mean concentration of Lake Bahire Giorgies was 0.665 mg/l in the water sample and the accumulation of Fe in fish muscles ranged from 34.9 mg/kg to 34.2 mg/kg and the average mean concentration was 34.5 mg/kg. The recorded concentrations were below the recommended limit of both EEPA (2003) and above WHO (2008) in water sample and also higher than WHO (2008) and FAO (2003) limits for fish and fish products.

Based on these results, the consumption of fish from Lake Bahire Giorgies has no risks on heavy metal related illness. The obtained results obviously showed that the concentration of the heavy metals (Zn and Cu) in water samples did not exceed WHO, EEPA but concentration of Fe was above the WHO and FAO standard limits and undetectable concentrations of Cd and Pb in both samples might be due to most metals being absorbed into suspended particulate matter and to be below the instrumental detection limit of the FAAS.

Fish are important aquatic organisms that are used as bio-indicators of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption. Bioaccumulation of metals in fishes takes place directly, from the water by gills and indirectly from food. Bioaccumulation of metal by an organism is the consequences of the interactions between the physiological factor (growth, weight loss, absorption, and accumulation), chemical factors (metal concentration, speciation, and bioavailability) and environmental factors (temperature, pH, water hardness, conductivity, salinity, and food) [15-17].

disorders, gastrointestinal tract, kidney, liver, blood vessels and central nervous system. Lead toxicity leads to anemia both by impairment of hemoglobin biosynthesis and acceleration of red blood cell destruction in human beings and inhibits some of the enzymes involved in energy metabolism, spinal deformities (FDREEPA and UNIDO, 2003). In all sampling sit the concentration of Pb did not detect in both water and fish samples these were might be the result of adsorption and accumulation of metals by suspendered solid, low instrument detection limits, and empty source of lead pollutions inter into the lake water.

**Cadmium (Cd)**

The concentration of Cd in the lake Bahire Giorgies at all sampling sit was not detected in both water and fish samples. The source of Cd in the environment could be attributed to the use of cadmium containing fertilizer, agricultural chemicals, pesticides and sewage sludge in farmland, due to natural mobilization of cadmium from the Earth's crust and mantles, such as volcanic activity including hydrothermal vents and weathering of rocks; current anthropogenic releases from the mobilization of cadmium impurities in raw materials such as phosphate minerals, fossil fuels and other extracted, treated and recycled materials might also contribute to the contamination of water but these sources did not the causes water pollutions.

**Comparison of the Levels of Metals within Water and Fish Samples**

The accuracy and precision of the results were checked by the aid of different statistical methods after the determination of the levels of metals in water and fish samples. The mean values were determined from triplicate analysis of each sample. The mean values determined were reported in terms of mean values (X) ± SD, for all the metals in this study.

Table 7: comparison of heavy metals in both water and fish sample.

| Metals | Samples concentration (mean ± SD | |
|---|---|---|
| | In water (mg/l) | In fish muscles (mg/kg) |
| **Pb** | ND | ND |
| **Cd** | ND | ND |
| **Cu** | 0.185±0.006 | 9±0.029 |
| **Zn** | 0.532±0.037 | 31.41±0.005 |
| **Fe** | 0.665±0.037 | 34.5±0.028 |

As shown from Table 7, the level of metals in water sample is lower than the level of metals in the fish sample. This could be due to bio-accumulation and bio-magnification of these metals in the body of the fish.

Studies also reported that metal concentrations in water samples were comparatively lower

In this study, the mean accumulations of Fe and Zn in the edible muscle of Nile Tilapia collected from Lake Bahire Giorgies ranged from 34.2 to 34.9 mg/kg and 31.2 to 31.5 mg/kg respectively. The levels of Fe and Zn in the muscle were not significantly different (P = 0.740 for Fe and P = 0.200 for Zn) among the sampling sites this shows that there is no variation of the accumulations of Fe and Zn in the edible muscle of Nile Tilapia fish throughout the lake. The accumulation of Pb and Cd in the edible muscle of Tilapia fish collected from Lake Bahire Giorgies ranged from below detection limit at all sit.

Generally, the concentrations of heavy metal from fish sample have higher than the water sample.

**Copper (Cu)**

The mean concentrations of copper found in Lake Bahire Giorgies 0.187 mg/l at sample one, 0.183 mg/L at sample two, and 0.186 mg/l at sample three. The average mean concentration of copper found to be 0.185mg/l in the water sample and the maximum average mean concentration of Cu (10.08 mg/kg) recorded in the fish sample collected at sample three which was the highest while the lowest mean concentration (8.1 mg/kg) was recorded in sample one.

The average mean concentration was 9 mg/kg the accumulation of Cu in fish muscles was lower than both EEAP (2003), FAO (2003), and WHO (2008) standard limits (Table 6). The concentration of Cu levels in sampling sites of Lake Bahier Giorgies could be attributed to agricultural activities in the catchment especially the use of fertilizers, fungicides, insecticides, and the sediment composition.

**Zinc (Zn)**

The mean concentrations Zn recorded in the present study were 0.563mg/L at sample sites one, 0.520 mg/l at sample sites two and 0.515 mg/l at sample three. The average mean concentration of Zn in this study area were 0.53 mg/l in the water sample and in Fish Muscle the highest mean concentration of Zn was 31.53 mg/kg at sample two and the lowest mean concentration of 31.2 mg/kg was measured at sample one. The average mean concentration accumulated was 31.41 mg/kg. These results were below EEPA (2003), WHO (2008) in water sample and from FAO (2003) WHO (2008) the recommended limit for fish and fish products.

**Lead (Pb)**

Lead is known toxic heavy metal to the peripheral system, reproductive system, immune system, cause blood and brain

than fish samples [8]. This might be due to the dilution effect.

In general, the distribution of the studied heavy metals in the lake water samples was below the permissible limits set by WHO and USEPA [15-18].

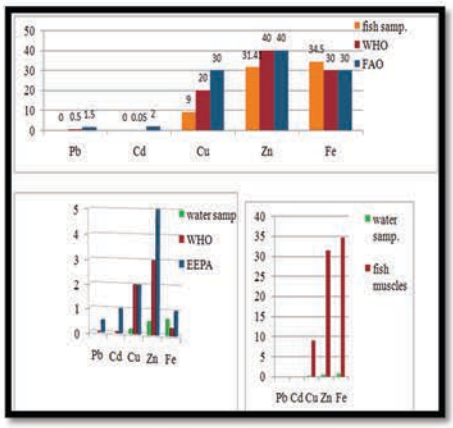


Figure 2: Compression of concentration between water and fish sample and its standard

Table 8 Summary of comparison of heavy metals in fish sample from Lake Bahrie Giorgies with reported values in fish samples from different Lakes in Ethiopia.

| No | Lake | Metals Concentration(mg/kg) | | | | | References |
|---|---|---|---|---|---|---|---|
| | | Fe | Zn | Pb | Cr | Cd | |
| 1 | Bahrie Giorgies | 34.5 | 31.4 | ND | ND | ND | This Study |
| 2 | Tana | 14.33-164.77 | 6.53-103.79 | BDL | 0.08-2.85 | BDL | [19] |
| 3 | Abaya | 105.94 | 14.02 | ND | 6.5 | 0.35 | [20] |
| 4 | Hashenge | 64.87 | 24.95 | 1.24 | 0.37 | 0.58 | [21] |
| 5 | Hawassa | 18.7-53.9 | 34.9-38.6 | 1.65-2.69 | - | 0.44-1.43 | [22] |

ND=Not Detected, BDL= Below Detection Limit

The comparison of the level of the studied metals in fish organs with values in reported works on different Lakes in Ethiopia is presented in Table 8. The level of Fe of this study (34.5mg/kg) was comparable with its level of fish from Hawassa (18.7-53.9 mg/Kg). Detected level of Zn (31.4 mg/Kg) was also comparable with its level of fish from Hawassa (18.7-53.9 mg/Kg). The toxic metals ( Pb, Cr and Cd) were not detected in this study. Whereas Pb was detected in Hawassa (1.65-2.69 mg/Kg) and Hashenge (1.24 mg/Kg). Cr was reported in three lakes (Tana, Abaya and Hashenge). The highest level of Cr was reported from Lake Abaya (6.5 mg/Kg). Generally, the level of heavy metals (Fe and Zn) from Lake Bahrie Giorgies is in between the minimium and maximium values reported in four lakes (Tana, Abaya, Hawassa and Hashenge). However, the level of Zn (31.4 mg/Kg) is higher than the reported values of two lakes (Abaya and Hashenge). This is mainly attributed to crop farming and anthropological activities around the Lake [19-22].

## Conclusion

In this study, the levels of the studied physicochemical properties in the lake water were found to be Temperature (18.6° c), pH (8.2), Electrical conductivity (82.7 μS/cm) and turbidity (348.6 NTU). From these parameters' turbidity of the Lake water has been above the standard limits. The concentration of heavy metals were also found in order of Fe (0.665 mg/L) > Zn (0.53 mg/L) > Cu(0.185 mg/L) but Cd and Pb did not detect in both samples in edible tilapia fish Fe (34.5 mg/kg) > Zn (31.41 mg/kg) > Cu (9 mg/kg). Except Fe, the concentrations of all metals detected were below the recommended limit from WHO (2008) EEPA and FAO in both samples. In general, the results reveal that heavy metals are more accumulated in fish muscle than in water sample due to bioaccumulation in nature.

All Physico-chemical parameters and heavy metals of the lake water were not significantly different ($p > 0.05$) among the three sampling sites and it showed uniform throughout sampling points due to anthropogenic water pollution. Among the detected metals, iron (Fe) showed a maximum accumulation in the edible muscle of Nile Tilapia fish and the maximum concentration found from a water sample in Lake Bahire Giorgies. Except Fe, the concentrations and accumulation of all metals recorded were below the recommended limit by FAO (2003), WHO (2008), and EEPA (2003) Good percentage recovery was obtained (86 to 96%) in a dry season the element Fe and Zn concentration in fish species, as well as water samples, were not significantly different at 95% confidence levels among different sites. However, the concentration of Cu was significantly different in the water and fish sample of the lake but the concentration of Zn and Fe were significantly correlated in both fish species and in the water samples.

Since the two toxic metals (Pb and Cd) are not detected for both Lake Water and the fish, the fish obtained from the Lake are safe and not dangerous for consumption. However, the lake is not suitable for fishery activity due to soil sedimentation that leads to a higher turbidity effect of the Lake water. Therefore, it is recommended that further studies should carry out for a continual assessment of the levels of pollutants.

## Acknowledgement

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# Critical Behaviors of a Two Dimensional Ising Model with no external Magnetic fields

Meseret Adane

Department of Physics, Debre Markos University, Debre Markos, Ethiopia

E-Mail: mesiadane47@gmail.com

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**Abstract:**

This study describes the critical behaviors of a two dimensional Ising model with different lattice sizes at a finite temperature using Monte Carlo (MC) simulation method and Metropolis algorithm in the absence of external magnetic fields. Only the nearest neighbors spin interaction is considered in each lattice site which prevents the system from ordering a full ferromagnetic or anti-ferromagnetic state. Thus, the critical behavior of the Ising Model below and above the critical point is observed. Average normalized magnetization and magnetic susceptibility were calculated. The phenomena of phase transitions of the Ising model are also explained. Thus, the critical temperature is determined in which the phase transition occurs.

**Keywords**: Ising Model; Monte Carlo Simulation; Critical Point; Phase Transition

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## Introduction

The Ising model was first proposed by Wilhem Lenz, who gave the problem to his student Ernst Ising who solved the model exactly in one dimension in his PhD thesis, but he was disappointed to see that there was no phase transition [1, 2]. About 20 years later, Lars Onsager modified and solved the Ising model exactly in two dimensions in the absence of an external magnetic field [3]. The Ising model concerned with the physics of phase transition, which occurs when a small change in a parameter such as temperature or pressure causes a large scale, qualitative change in the state of the system.

Ising introduced a model is consisting of a lattice of spin variables $S_i$, which can only take the value+1 (spin up) and -1 (spin down). Every spin interacts with its nearest neighbors as well as an external magnetic field [4, 5]. The simplest system that exhibits a phase transition is the Ising model. The Ising model is the most studied model in statistical physics. It is an extremely interesting model despite its simplicity. It is highly applicable in different areas. The three common applications are to magnetism, fluids (lattice gas) and to binary alloys [6]. Consider a square Ising lattice with size L as shown in figure 1.1 below.

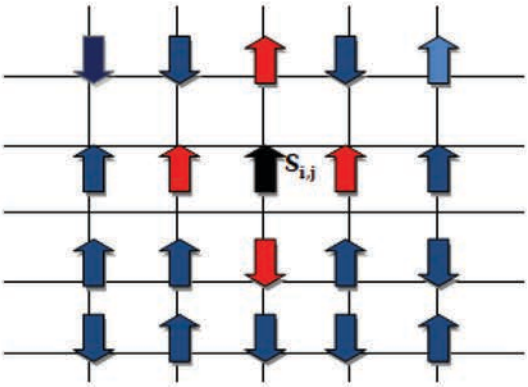


**Figure 1.1** Model of a 2D square Ising lattice with sides L.

For a 2D Ising model there are four nearest neighbors spin interaction. From figure 1.1 let $s_{i,j}$be the initial spin state at lattice coordinates i and j having either spin up or

spin down, $s_{i,j}$= ±1.The Black arrow represents the initial spins$s_{i,j}$ and the red represents the nearest neighbor spins at (i±1, j) and (i, j±1) coordinates. The Hamiltonian or total energy of the system in a particular state $\{S_{i,j}\}$ is given by [7]:

$$H(\{S_{i,j}\}) = J \sum_{\{(i\pm1,j),(i,j\pm1)\}} S_{i,j}\left(S_{i+1,j} + S_{i-1,j} + S_{i,j+1} + S_{i,j-1}\right) \quad (1)$$

Where, the sum runs over all nearest neighbors of $s_{i,j}$ for the given square lattice of linear dimension L with periodic boundary conditions. J is the coupling constant which specifying the strength of interaction. We can see that the effect of J on the behavior of the spins [8, 9]. When J > 0, neighboring spins prefer to be parallel and are called the ferromagnetic model. When J < 0, neighboring spins prefers, aligns anti-parallel and is called anti-ferromagnetic model. For the lattice size L and dimension D the number of spins in the lattice size is given by: $N = L^D$ and the possible ways to order the spins equal to $2^N$. Example, for a square Ising lattice with size L, there are $N = L^2$ spins. The Ising model is usually studied in the canonical ensembles. In canonical ensemble, the probability of finding a particular configuration $S_{i,j}$ is given by [9]:

$$P(\{S_{i,j}\}) = \frac{1}{Z(\beta)} exp^{[-\beta H(\{S_{i,j}\})]} \quad (2)$$

Where $\beta = \frac{1}{k_B T}$, T -temperature, $k_B$ is Boltzmann constant and

$$Z(\beta) = \sum_{i,j=1}^{N} exp^{[-\beta H(\{S_{i,j}\})]} \quad (3)$$

Where, $Z(\beta)$ is partition function

**Ferromagnetism of Ising Model**

Ferromagnetism is the property of a paramagnetic material to retain spontaneous magnetization as the external magnetic field removed. At low enough temperature, all spins in the two dimensional Ising model will cooperate and spontaneously align themselves even in the absence of the external magnetic field. This phenomenon is called spontaneous magnetization. It is a macroscopic quantitative measurement of the spin fluctuation of the system is referred to as spontaneous magnetization [9, 10]. The magnetization is given by:

$$M = \sum_{i,j=1}^{N} \{S_{i,j}\} \quad (4)$$

and average magnetization is given by:

$$\langle M \rangle = \frac{1}{n} \sum_{i,j=1}^{N} \{S_{i,j}\} \quad (5)$$

Where, n is number of spin configurations of lattice. The mean normalized magnetization is given by [10]:

$$m = \frac{\langle |M| \rangle}{N} \quad (6)$$

Where, $m$ is mean normalized magnetization per spin and N is number of spins on the lattice sites. Since the magnetization is proportional to $\langle S_{i,j} \rangle$, there is a spontaneous M > 0 at low temperature and the system is ferromagnetic. At high temperatures, the disordering effect of the temperature dominates and the system is paramagnetic with M = 0. The transition between these phases is abrupt, at critical temperature, $T_C$. This is an example of a second order phase transition. For this transition, the spontaneous magnetization is known as the order parameter. M has non zero values when the system is in the ferromagnetic phase [11, 12]. The relation between magnetization and interaction energy (couplings constant J) for different lattices have been studied by Dhia`a Khalid K. [13]. The researcher calculated both positive and negative magnetization. But in our study we calculated the mean normalized magnetization (only positive value) for different lattice sizes and we plotted the temperature dependent mean normalized magnetization graph to show the phase transition.

A measure of how much magnetization is induced by the application of magnetic field is called magnetic susceptibility (χ). Susceptibility can be calculated using the equation [4]:

$$\chi = \frac{1}{NT}[\langle M^2 \rangle - \langle M \rangle^2] \quad (7)$$

Many researchers tried to study the critical behavior of a 2d Ising model by fixing the dimensionless parameter β in terms of $\frac{J_C}{k_B T}$. But in this study we are interested to

investigate the effects of the relationship that we assumed on the critical behavior, particularly the effects of the inverse relationship of temperature with the couplings constants on mean normalized magnetization and susceptibility of our model. But, in this paper, temperature $T \sim \frac{1}{J_C}$, where, $J_C$ is coupling constant. Hence, equation (7) can be written in terms of couplings constant (Jc), magnetization (M) and number of spins in the lattice sites (N) as follows.

$$\chi = \frac{J_C}{N}[\langle M^2 \rangle - \langle M \rangle^2] \quad (8)$$

The temperature dependence of the magnetic susceptibility of the 2D Ising model for different lattice sizes was studied and calculated by Anders W. Sandvik [14], using finite- size scaling power law method. The maximum value of the susceptibility for a given system size L should be: $\chi(L)L^{\gamma/v}$. He plotted magnetic susceptibility versus temperature graph for different lattice sizes and showed the peak value of magnetic susceptibility grows very rapidly with L. Here the shift in the peak temperature with L can also be clearly seen. We also have calculated magnetic susceptibility for a 2d ising model for different lattice sizes using equation (7) or (8).

Dhia`a K. Khudier and Nabeil A. Fawaz [15], also have studied magnetic susceptibility against the temperature ($\frac{J}{kB}$) for different lattice sizes (16x16,30x30 and 55x55) for case of zero external field (B=0) but they did not described the clear relationship between the value of magnetic susceptibility the lattice size L. In addition, G.A. Alves , M.S. Vasconcelos, and T.F.A. Alves [16], calculated the susceptibility as a function of temperature (T in units of $\frac{K_B T}{J}$ ) for different lattice sizes L. The values of L obey the Octonacci sequence. The susceptibility diverges at Tc in the large lattice size limit suggesting a second order phase transition. The same effects are observed as Anders W. Sandvik's result that we have discussed before.

## Methods

### Simulation Schemes

We considered 2d Ising models in a finite lattice site on a square box of side L and with four different lattice sizes. Hence we used the lattice sizes, L = 80, 100, 120, and 200. From each lattice size, $10^3$states of spin configuration are taken randomly for samples and only the nearest neighbors spin

interactions are considered. We used FORTRAN code program and FORTRAN 90/95 code compiler of Ubuntu software operating system to carryout simulations. Metropolis algorithm is used for the energy test. For the chosen initial spin, the old energy before flipping and the new energy after flipping were calculated using equation (1) by applying the nearest spins interaction. Then, we determined the change in energy between the new and old energies which is important to find the Boltzmann factor. By comparing the Boltzmann factor with some random number generator which lies between 0 and 1 to accept or reject the move. We have done the simulations with MC warm up steps of $4 \times 10^4 \times L^2$ and the moves of MC time steps taken as $5 \times 10^4 \times L^2$ where L is the lattice size. We applied the periodic boundary conditions and initialization conditions of the order parameter. Hence we initialized average magnetization $\langle M \rangle_0 = 0$ and the coupling constant $Jc = 0$. We also determined the initial spin configurations for each MC move through the lattices and according to equations (6) and (8), we carried out simulations by using Monte Carlo (MC) method to calculate average normalized magnetization and magnetic susceptibility as a function of temperature for every Monte Carlo time step and every Monte Carlo warm up step for each lattice size. Here one unit of time corresponds to one complete move through the lattice, so that every spin had the opportunity to flip once during each time step. These mean values are calculated for 15 iterations.

We also performed simulations to study the spins fluctuation by warm up the models for each lattice size within a given MC time steps and by changing the couplings constant (Jc) and temperature (T). In these simulations, we assumed the Boltzmann's factor $k_B$= 1 unit and the temperature is in the dimensionless parameter of $\frac{1}{Jc}$and in the inverse relationship with Jc. Therefore, we assumed the dimensionless temperature is inversely proportional to the couplings constant. That is, temperature $T = \frac{1}{Jc}$.

## Results and Discussion

### Spin Configurations as a function of Temperature

Now let us explain what we have seen about spin configurations for a square Ising lattice with different lattice sizes above critical temperature ($T > Tc$),at critical

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temperature ($T \approx Tc$) and below critical temperature ($T < Tc$).

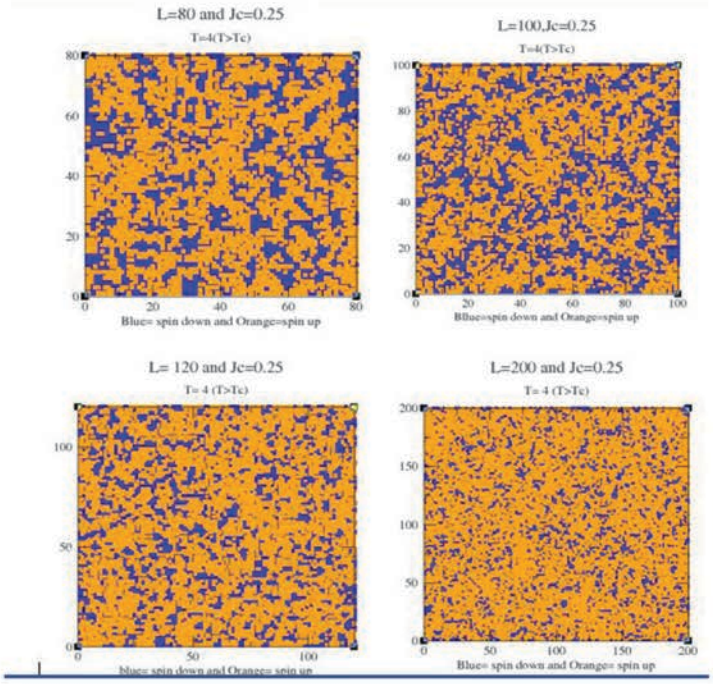


**Figure 3.1** Snapshots of Spin configurations of a 2d Ising models in a finite square box for different lattice sizes above the critical temperature, T = 4 or $Jc = 0.25$.

The Fig 3.1 shows snapshots of spin configurations of a 2d Ising model for different lattice sizes above the critical temperature ($T > Tc$) at T=4 or $Jc = 0.416$. Blue represents down spins and orange represents up spins. It shows that proceeding to higher temperatures, we defined that T=4, the fluctuation decreases in magnitude in relative to the fluctuation around the critical temperature which is $Tc \approx 2.4$. There is no dominance of one spin type because the neighbors of any particular spin are not randomly aligned above the critical temperature. Hence the fluctuation pattern is independent of lattice size. Here the spins are aligned themselves under a paramagnetic state (disorder phase). In relative to the low temperature phase, thermal effects dominate at high temperatures above critical points.

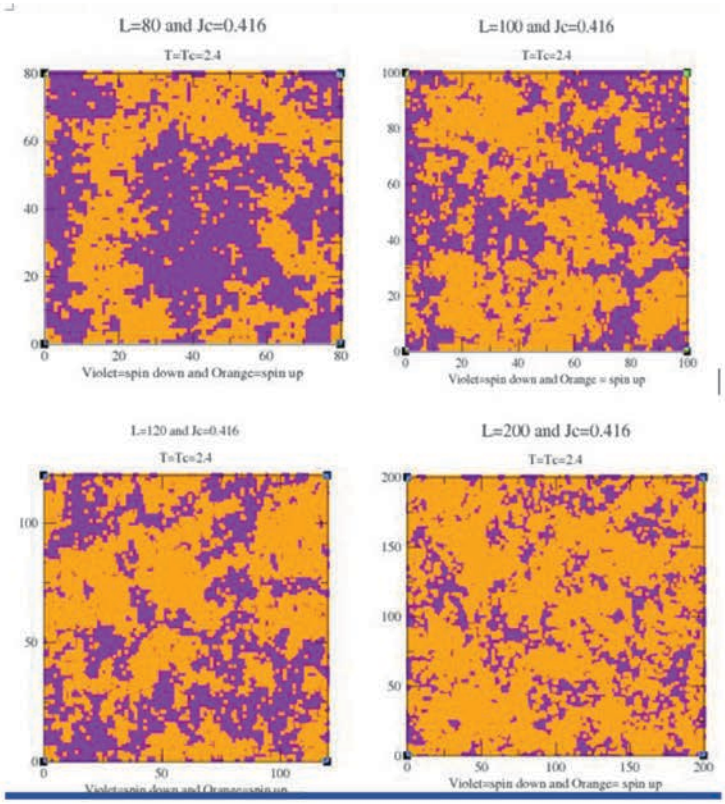


**Figure 3.2** Snapshots of spin configurations of a 2d Ising models in a finite square box for different lattice sizes at the critical temperature, Tc = 2.4 or Jc = 0.416

Figure 3.2 shows snapshots of spin configurations of a 2d Ising in a finite square box with different lattice sizes at the critical temperature$Tc = 2.4$.Orange color represents up spins and violet represents down spins. When we increase the lattice sizes of the models in the simulations, the results would look self-similar. For all lattice sizes, the system has an abrupt change of spin fluctuation at $Tc$and the domains are created. Therefore, there is no a clear dominance of one spin around $Tc \approx 2.4$. That means the winner of spin alignment is unknown. The disorder of spins increases relative to at a low temperature below the critical point. As the temperature increases to around the critical temperature, the magnitude of fluctuation increases and tends to maximum. This means that the degree of ordered decreases from its value at low temperature below $Tc$.

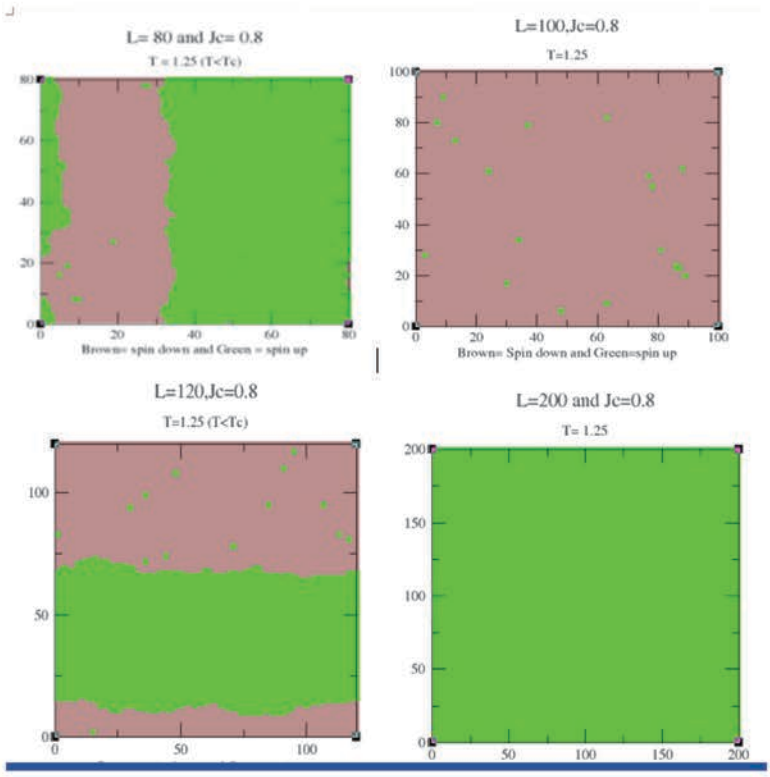


**Figure 3.3** Snapshots of spin configurations of a 2d Ising models in a finite square box for different lattice sizes below critical temperature at T = 1.25 or Jc = 0.8.

Figure 3.3 shows configurations of a square Ising models with different lattice sizes below critical temperature. Brown represents down spins and green represents up spins. In this case there is a clear dominance of one type spins. For L=100, majority spins are aligned in the same direction which are pointing spin down with the minority up spins. Minority spins have nearest neighbors majority spins only, and these pairs are linked by anti-ferromagnetic bonds. Similarly, for L=200, at the given temperature and couplings constant, majority spins are pointing up with minority spins pointing down. Because, in the first case, L=100, we supposed that, initially all (or most) spins pointing up in a ferromagnetic phase. At T=1.25 and $Jc = 0.8$, most spins pointing down. This means, thermal fluctuation energy is dominated than the strength of the spin-spin interaction energy. On the other hand, in the case of L=200, at T=1.25 and $Jc = 0.8$, most spins pointing up. This means, the strength of the spin-spin

interaction energy dominated than thermal fluctuation energy.

On the other hand, for L=80 and L=120 Ising models, the large domain walls are created. As a result, we observe a 'trapped' surface which is bounded by the domain walls. The spins at the right edge of the grid are constrained to point in the up direction and majority spins point in down direction at the left edges of an 80 × 80 Ising model. Similarly, the horizontal 'trapped' surface is formed by the large domain walls for L=120 Ising lattice. At the middle of the model, all spins are aligned up direction and either edges of the model, majority spins are aligned in opposite direction with minority up spins which paired by anti-ferromagnetic bonds with the majority down spins. For all grid sizes the majority spins are paired each other by ferromagnetic bonds. Unnar B Arnalds *et al* [16], were studied the typical spin configurations of a 2d Ising model at different temperatures in the unit of $\frac{J_c}{K_B}$ which is the main difference compared to our work and they have taken snapshots of the spin configurations at different temperatures below critical temperature (T < $Tc$), at critical (T≈ $Tc$) and above critical temperature (T > $Tc$). In our work the same effects are observed except the 'trapped' surfaces which formed by the large domain walls for L=80 and L= 120 lattice sizes at low temperature below the critical temperature (T < $Tc$). In addition, Anders W. Sandvik [14], showed the snapshots of Ising spin configurations obtained after three consecutive Monte Carlo steps at three different temperatures, T=2.0, 2.5, 4.0. The configuration generated at T = 2.5, is approximately the temperature at which the size of the ordered domains becomes comparable with the system length for the system size L = 32 considered (this temperature can be taken as a critical temperature $Tc$ for a given system size, which is higher than the value in the thermodynamic limit, known exactly from Onsager's solution to be $Tc = 2/ln\left(1 + 2\sqrt{2}\right) \approx 2.269$). But this study determines the critical temperature $Tc = 2.4$ which is closer to the Onsager's exact solution compared to Anders W.Sandvik's result.

**Average Normalized Magnetization**

Magnetization is a macroscopic quantitative measurement of the spin fluctuation of the system. It depends on average spins$\langle S_{i,j} \rangle$. We calculated average normalized magnetization is using equation (5).

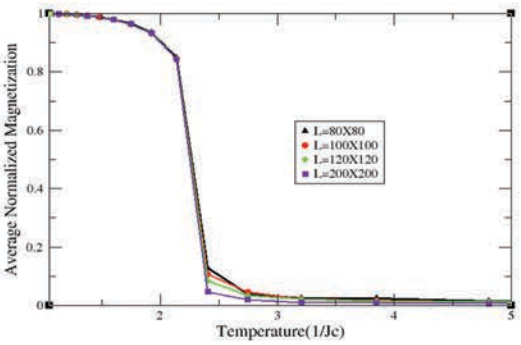


**Figure 3.4** Mean normalized magnetization as a function of temperature for different lattice sizes.

Figure 3.4 shows the average normalized magnetization versus temperature graph for different lattice sizes. From this we can see that, the average normalized magnetization increases rapidly in the region (interval) of $T \approx 2 \longrightarrow 2.5$. Above the specified temperature interval, the mean normalized magnetization drops to zero. On the other hand, it does not drop to zero below the given temperature interval. This means average normalized magnetization changes from 0 above the interval of temperature$T \approx 2 \longrightarrow 2.5$ to a finite value below the interval. At low temperatures below the given interval of temperature, the average normalized magnetization stays close to the saturation value corresponding to all of the spins being parallel. We can observe the signs of phase transition since the magnetization increases rapidly in the region around$T \approx 2 \longrightarrow 2.5$. For all lattice sizes, the models are the ordered ferromagnetic below the interval $T \approx 2 \longrightarrow 2.5$and disordered paramagnetic above the given interval. Hence there is a phase transition from ferromagnetic to paramagnetic phase abruptly at the temperature around T $\approx$ 2.4.At low temperatures, the interaction between the spins seems to be strong; the spins tend to align with another. In this case, the mean normalized magnetization reaches its maximal value, $\langle m \rangle$= 1, according to its formula given by equation (6), the magnetization exists even if there is no external magnetic field.

However, other physicists and mathematicians pursued the model further, and it has continued to be a significant model in statistical mechanics ever since. Nearly twenty years after Ising abandoned the problem, Kramers and Wannier [17], showed that the two dimensional model displayed phase transitions at the Curie temperature, $Tc = 2/ln\left(1 + 2\sqrt{2}\right) \approx$ 2.269, using high and low temperature expansions of the model.

Dhia Khalid Khudier and Nabeil Ibrahim. Fawaz [15], showed the impacts of the magnitude (size) of the lattice on the transition of the stage for different lattice

sizes (L×L=4, 8, 16, 32) without effect of the magnetic field (B=0). Thus, the bigger is the lattice, the faster is the demagnetization which means, the faster occurrence of phase transition. Therefore, in our study we analyzed the behavior of magnetization and

**Magnetic Susceptibility**

The magnetic susceptibility of a two dimensional Ising model is calculated in the phase transition by increasing the lattice size ranging from L=80 to L =200. Hence, the lattice with size L= 80, changes its phase rapidly compared to L= 200 at the critical temperature $T_C = 2.4$.

simulations by using equation (7) for different lattice sizes.

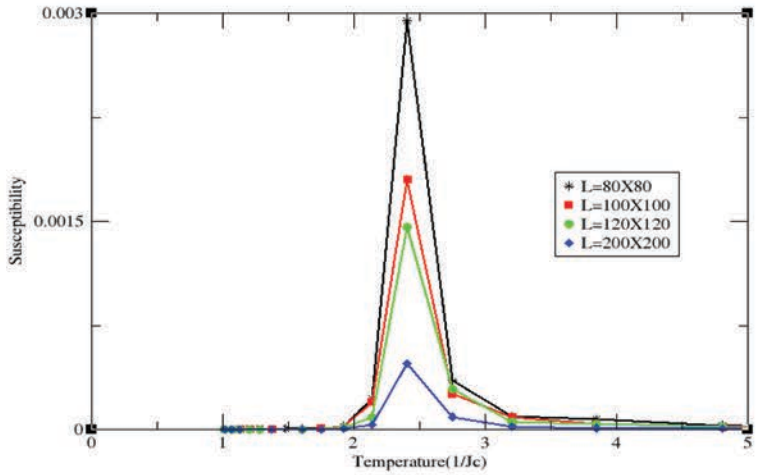


**Figure 3.5** Magnetic susceptibility as a function of temperature for different lattice sizes

Figure 3.5 shows the size dependence of susceptibility curve as a function of temperature, computed by the Metropolis algorithm. The susceptibility is the order parameter which shows the point of phase transition in which the critical point occurs. Hence from the graph we observe the critical point occurs at a temperature of $Tc \approx 2.4$ which corresponds to the maximum of susceptibility for different lattice sizes. For all lattice sizes, the susceptibility increases as T goes Tc to the right and to the left. The larger the system size gives rise to the smaller susceptibility and the larger fluctuation. From this we can see that, as the system size increases, the corresponding height of the peak of the susceptibility curve decreases. The sharp peak appears at the critical temperature around $Tc \approx 2.4$ regardless of the lattice size. This means that, the critical

temperature is insensitive to the lattice size. From figure 3.5, we observed that, the peak value of magnetic susceptibility grows very rapidly as the lattice size L decreases and shift in the peak temperature or critical temperature with L and it is insensitive to the lattice size.

**Conclusion**

Based on the simulations the critical point appears at the value of temperature around $Tc \approx 2.4$ which is slightly larger than the Onsager's critical temperature $Tc = 2.269$ and the value of the critical temperature is independent (insensitive) of lattice sizes. Around critical temperature ($T = Tc \approx 2.4$), there is abrupt change of spin configurations and the domain walls are created with the unknown winner of spin and the disorder of spins increases relative to at a low temperature below the critical point. Therefore, as the temperature increases to around the critical temperature, the magnitude of fluctuation increased. Also we studied the behavior of the model below and above the critical point in comparison with the critical behavior for different lattice sizes. At low temperature below $Tc$, majority spins are ordered and aligned themselves in the same direction and hence they are ferromagnetic. Besides of this, at high temperature above $T_C$, the spins are in disordered phase and hence they are paramagnetic.

The average normalized magnetization and magnetic susceptibility as a function of temperature

are also calculated for different lattice sizes. Hence the mean normalized magnetization is increases rapidly in the interval around $T \approx 2$ to 2.5. If the temperature goes to zero below this interval, the mean normalized magnetization does not drop to zero and it is tends to unity. Besides this, the magnetization is vanishes for the interval $T \approx 2$ to 2.5. This means that there is the order phase transition from ferromagnetic (below $T \approx 2$ to 2.5) to paramagnetic phase (above $T \approx 2$ to 2.5). On the other hand, the maximum of susceptibility increases as the system size decreases and approaches to $Tc$. The susceptibility becomes maximum at the critical point around $Tc \approx 2.4$. This means that, the sharp peak of the susceptibility curve appears at the critical point $Tc$ regardless of the lattice size and the critical temperature is insensitive to the lattice size.

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**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES**

**DEBRE MARKOS UNIVERSITY**

**DEBRE MARKOS, ETHIOPIA**

**+251 588914909**