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Effect of Olive Fruit Fly (*Bactrocera oleae* (Rossi)) on Selected Quality Indicators of Virgin Olive Oil from Palestine

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Thesis Approval

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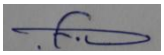
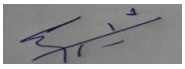
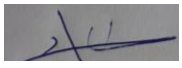
1. Prof. Fuad Al-Rimawi: Committee Member, Signature: 

2. Prof. Jehad Abbadi: Co-supervisor, Signature: 

3. Dr. Ziad Ayyad: Internal Examiner, Signature: 

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Dedication

I dedicate this thesis...

To my beloved wife, to my dear children, and to my brothers and sisters

To my beloved family big and small who supported and encouraged me through all the stages.

To my friends, my colleagues

To everyone who helped me complete this study

With respect and love.

Declaration

I certify that this thesis submitted for the degree of master is my own research, expect where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Signed: Ahmad

Ahmad "Mhammad Ali" Qrinawi

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First, I thank God for helping me during my studies and completing this study. I would like to express my gratitude and appreciation to everyone who contributed to the success of this study.

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Finally, my warm appreciation to my family, respectful parents, and siblings who have continuously supported me throughout my educational journey. I also wish a special thanks to my friends - for their supportive role and patient attitude, it was most welcome.

Abstract

This study's objective is to assess how olive fruit flies' infestation affects various olive oil quality indicators, including antioxidant activity, total phenolic and flavonoid content, Acidity peroxide value, K232, and K270. Olive fruit flies' punctures on the fruits that had an active infestation could be seen. In comparison to healthy oil samples (from olive fruits not attacked by the fly), the study's findings showed an increase in the acidity , peroxide, and K values (K232 and K270) of olive oil from samples of olive fruits. This suggests that an olive fly infestation has caused a decrease in the quality of olive oil. This is explained by the accelerated oxidation and hydrolytic degradation, which are encouraged by having exit holes that allow oxygen and microorganisms to affect the olive pulp. The results also revealed that the total phenolic and flavonoid contents of olive oils made from unhealthy olive fruits were lower than those made from healthy fruits, which further suggests that the oil is of lower quality. These polyphenolic compounds act as antioxidants and are crucial to the stability and shelf life of olive oil. Similar to this, a decline in the antioxidant activities of olive oils from unhealthy fruits (reflected by FRAP and DPPH assays) also indicated a decline in the olive oil's quality. This suggests that, in the event of an olive fly attack, the category of olive oil may change. For example, oil from unhealthy fruits may be downgraded from extra version to version olive oil.

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Chapter 1: Introduction:

1-1 Background

The cultivation of the olive tree (Olea europaea L) holds a significant global importance due to its production of high-quality edible oil that boasts exceptional nutritional and health benefits. According to the International Olive Council (IOC) in 2016, the worldwide production of olive oil was estimated at 3,225,500 tons, . On average, Palestine produces 15,500 tons of olive oil annually, contributing to the global production. The Mediterranean countries are the primary producers and consumers of olive oil, with the European Union (EU) leading the production. As shown in Figure 1.1, the production of olives and olive oil is highest in the Mediterranean countries (IOC, 2016).

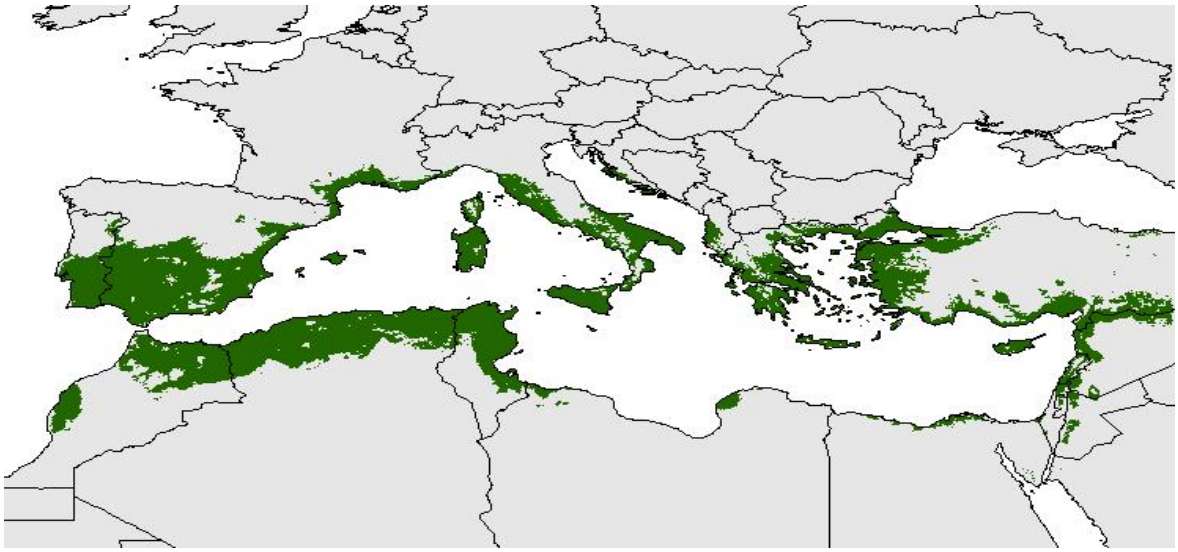


Figure1.1: Mediterranean countries olive and olive oil producers.

The olive tree holds great cultural significance in Palestine, serving as a symbol of the people's deep-rooted connection to their land and heritage. The region's favorable soil and climate conditions have contributed to the production of some of the world's finest olive oils, as recognized by the United Nations in 2008. The International Olive Oil Organization (2008) reports that out of the 750 million olive trees grown globally, 95% are located in the Mediterranean region.

Olive cultivation is a critical component of the Palestinian agricultural sector, accounting for approximately 45% of it. The fruit of the olive tree is primarily utilized for the production of virgin and extra virgin olive oil. Salimian et al (2009) reported

that olive tree cultivation covers an estimated 100,000 hectares of agricultural land, constituting 45% of the total agricultural land in Palestine. In the West Bank and Gaza, there are 270 oil press plants that can extract 32-35 thousand metric tons of olive oil annually. Olive oil production represents 93% of the olive harvest in Palestine, contributing to 18% of the total agricultural economy. The average cost of producing one liter of Palestinian olive oil for farmers is approximately 2.3 US dollars, whereas the retail selling price ranges between 6-7 US dollars.

Olive oil offers numerous established health benefits, owing to its rich content of healthy monounsaturated fats and antioxidants. The oil is renowned for its potent anti-inflammatory properties, which can help protect against heart disease. Research has also suggested that olive oil consumption may lower the risk of type 2 diabetes. Moreover, the antioxidants found in olive oil possess both antibacterial and anticancer properties.

Chapter 2. Literature review

2-1 Quality of olive oil

2.1.1**. Olive oil** The term "extra virgin olive oil" refers to oil that is exclusively extracted from the fruit of the Olea europaea L tree and does not contain any other oils. The oil is classified and marketed based on specific definitions and designations.

2.1.1.1. According to the IOOC in 2015, virgin olive oils are derived from olive fruits solely through physical or mechanical methods without any changes made to the oil. This process does not include any treatments that could modify the composition of the oil, particularly those that involve heat, and m.ay involve decantation, filtration, or centrifugation, among other methods.

Virgin olive oils fit for consumption as they are include:

a. To be classified as extra virgin olive oil, the oil must adhere to specific requirements, such as having a free acidity percentage of no more than 0.8 grams per 100 grams, no negative organoleptic attributes, positive sensory qualities determined by a taste panel, a peroxide value not exceeding 20 milliequivalent peroxide O2 per kg oil, and conformity with all standards in its category.

b. Virgin olive oil is produced from the olive fruit using solely mechanical or physical methods without undergoing any modifications. The free acidity percentage of this oil must not exceed 2 grams per 100 grams, expressed as oleic acid, and must meet other specified standards for this category.

c. Ordinary virgin olive oil is a type of olive oil that is extracted from the olive fruit using physical or mechanical methods without undergoing any changes to the oil. This category of oil has a maximum free acidity level of 3.3 grams per 100 grams expressed as oleic acid and complies with all other standards set for this category, as established by the IOOC in 2015.

2.1.2. **Olive-pomace oil** Olive-pomace oil is produced by utilizing solvents or physical methods such as heat and pressure to treat the residue left after the extraction of virgin olive oil, which is known as olive pomace. The resulting oil is then blended with virgin olive oil after refining, and is frequently utilized for cooking purposes. It should be noted, however, that olive-pomace oil is not considered to be of the same high quality as virgin olive oil and is typically employed as a less expensive alternative for cooking and frying. The IOOC acknowledges this oil as a separate category, and has established specific quality standards for its production and distribution. (IOOC, 2019).

2.2. Composition of olive oil

Olive oil comprises two main components, the major and minor components. Triglycerides constitute the majority of the oil, making up around 98-99% of it. The minor components, which make up the rest, consist of free fatty acids, phospholipids, sterols, tocopherols, pigments, and phenolic compounds. These minor components play a significant role in determining the oil's taste, aroma, and nutritional value, as well as the health benefits associated with its consumption. (Garcia-Oliveira et al., 2021)

2.2.1. Major components:

The major component of olive oil is the saponifiable or glyceride fraction, which comprises about 98% of its weight. It consists mostly of triacylglycerols and includes six primary fatty acids: oleic acid, palmitoleic acid, palmitic acid, stearic acid, linoleic

acid, and linolenic acid. Oleic acid, the most abundant fatty acid in virgin olive oil, typically makes up 68-81.5% of the fatty acid profile, as noted in studies by Houshia et al. (2014) and Hamid et al. (2010). These fatty acids play a crucial role in determining the sensory properties and nutritional value of olive oil.

2.2.2. Minor components:

The minor components of olive oil constitute a small portion of the oil, approximately 1-2% of its total weight, and are composed of more than 230 distinct compounds. These include non-glyceride esters such as waxes, alcohols, sterols, hydrocarbons, pigments, tocopherols, phenolic compounds, and volatiles. Although only a few of these compounds have been identified as bioactive and associated with health benefits, they contribute significantly to the oil's taste, aroma, and nutritional value. Studies conducted by Covas et al. (2006) and Ramirez-Tortosa et al. (2006) confirm this.

2.3. Olive oil quality indices

2.3.1. Acid Value

The acid value of a fat or oil is a measure of the free fatty acid content, determined by calculating the amount of potassium hydroxide required to neutralize these acids in one gram of the sample. This value is crucial in assessing the quality of the oil, as free fatty acids are generally produced during the decomposition of oil glycerides, indicating rancidity or degradation. Typically, the acid value is expressed as a percentage of free fatty acids calculated as oleic acid. (Hamid, F., & Hamid, F. H.2016).

2.3.2. Peroxide Value

Lipid oxidation is an ongoing process that involves the creation of hydroperoxides, which arise from the oxidation of unsaturated fatty acids such as oleic, linoleic and linolenic acids. Initially, the rate of hydroperoxide production is higher than their rate of decomposition, which eventually leads to the buildup of peroxides and oil rancidity. As a result, the peroxide value (PV) is a critical measure of the initial stages of oxidative change because it determines the total hydroperoxide content and serves as

a quality indicator during the production and storage of fats and oils (Antolovich et al., 2002).

2.3.4. K_{232} and K_{270}

The UV spectrum is a measurement of the absorption of fatty acids through electronic means, and the 230-270 nm band is significant for indicating absorption when unsaturated fatty acids undergo autoxidation, which results in conjugated dienes and trienes. K232 and K270 are measurements of absorbance at 232 nm and 270 nm, respectively, and are commonly utilized in olive oil quality control to detect oxidation and rectified oil adulteration. This method provides information about the extent of oxidation that occurs during production and storage, resulting in primary and secondary products. Research conducted by Mignan et al. (2012) and Angerosa et al. (2006) indicates that K232 and K270 are effective indicators of product oxidation and adulteration. Additionally, studies by Afaneh et al. (2013) and Al-Rimawi, F., et al. (2014) have confirmed the usefulness of this method.

2.4. Phenolic compounds

Phenolic compounds are a class of secondary metabolites that contain an aromatic ring and a hydroxyl group on a benzene ring. They are found abundantly in plants and are derived from the shikimate pathway and phenylpropanoid metabolism. This group of chemicals is highly diverse and includes a range of substances that possess an aromatic ring and one or more hydroxyl groups (Ryan et al, 2002).

In scientific literature, the terminology used to refer to phenolic compounds may vary depending on the specific area of study. For instance, terms such as phenols, polyphenols, and biophenols may be used. However, in the context of Olea europaea L. matrices, the preferred terms are olive phenols or olive phenolic compounds. This information is based on the study conducted by Uccello in 2000.(Houshia & Qutit, 2014).

2.4.2. Functions of polyphenolic compounds and antioxidants in olive oil

a. Nutritional quality: Studies have shown that the presence of phenols in olive oil can enhance its nutritional quality due to their high antioxidant activity (El Riachy et al., 2011).

b. Health benefits of olive oil phenols: Phenols found in olive oil have been studied extensively for their positive effects on health. These benefits include their antioxidant, antithrombotic, antihypertensive, and anti-aging properties, as well as their potential to protect against diseases such as cancer, neurodegenerative disorders, and cardiovascular diseases, as reported by Vissers et al. (2004).

c. Sensory quality: Phenols, in conjunction with volatiles, are essential in shaping the organoleptic attributes of olive oils, which influence their unique and exquisite taste and aroma that are greatly appreciated by consumers (Servili et al., 2009).

d. Oxidative stability: Phenols are crucial in preventing the oxidation and prolonging the shelf life of virgin olive oil. These compounds possess unique properties that enable them to scavenge radicals, transfer hydrogen atoms, and chelate metals, thus making them effective in inhibiting lipid oxidation during the early stages. (Jerman, 2014).

2.5. Phenolic compounds groups

It is possible to classify polyphenols into various categories based on the number of phenol rings and the structural elements connecting these rings ((Esterbauer, H. et al,1990). The most significant categories of polyphenols are phenolic acids, flavonoids, stilbenes, and lignans. In olive oil, different types of polar phenolic compounds are present, as shown in Figure 1.2, and examples of representative molecular structures are also provided.

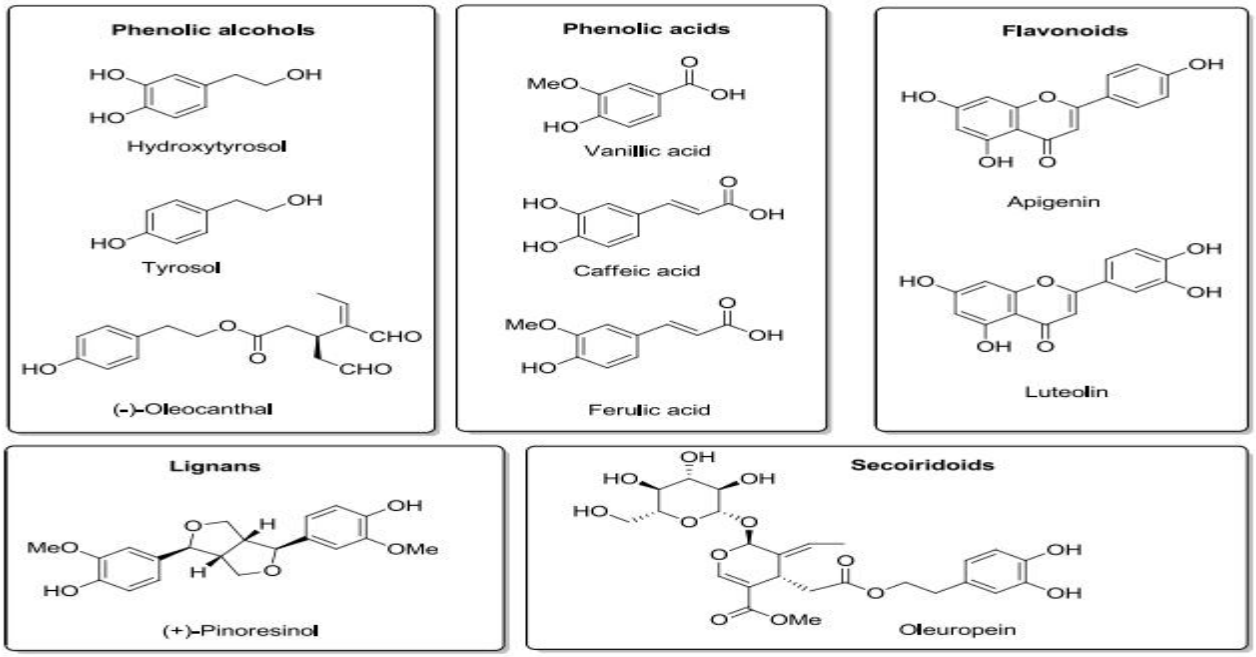


Figure.1.2: The different classes of polar phenolic compounds present in olive oil with molecular structures of representative examples (Angerosa, et al. 2006).

2.6. Oxidation of olive oil

Oxidation is a chemical process that involves electron transfer from one substance to an oxidizing agent and can lead to the formation of free radicals. Free radicals are unstable and highly reactive molecular species that contain an unpaired electron in an atomic orbital. This property is shared by most radicals, and they can cause damage to cells by initiating chain reactions (Hamid et al, 2010; Lobo et al, 2010).

To maintain equilibrium, the body produces antioxidants that neutralize the free radicals generated during internal processes. However, when the balance is disturbed, oxidative stress can occur, disrupting normal cell functions and leading to cell death. This imbalance can be caused by overproduction of reactive species and inadequate antioxidant defense mechanisms, resulting in various chronic diseases, such as cancer, diabetes, and neurodegenerative and cardiovascular disorders (White et al, 2014).

2.7. Antioxidants in olive oil

Antioxidants are compounds that can effectively prevent or slow down the oxidation of substances that are susceptible to oxidation, even when present in smaller concentrations than the oxidizable substances (Antolovich et al, 2002).

Studies conducted both in vitro and in vivo have shown that antioxidants, including vitamin E (alpha-tocopherol), carotenoids, and phenolic compounds, including simple and complex phenols, have antioxidant properties and can help prevent certain diseases while slowing down the aging process. Virgin olive oil, which is abundant in these antioxidants, is particularly effective in safeguarding against free radical damage and inhibiting cancer formation. (IOOC, 2015).

2.7.1 Mechanism of action of antioxidants

Free radicals can attack different types of biomolecules in the body, with polyunsaturated fatty acids (PUFAs) in cell membranes being particularly susceptible to damage. This damage to PUFAs, known as lipid peroxidation, is especially harmful as it is a self-propagating chain reaction. When a PUFA is oxidized, it generates a fatty acid radical (L˙) (Equation 1), which rapidly reacts with oxygen to form a fatty acid peroxyl radical (LOO˙, Equation 2). These peroxyl radicals initiate further chain reactions that lead to the oxidation of PUFA molecules and the formation of lipid hydroperoxides (LOOH) (Equations 3 and 4). These hydroperoxides can then further decompose into more radical species (Esterbauer et al, 1990).

2.7.2 Methods of evaluation of antioxidant activity

2.7.3 DPPH:

The DPPH assay is a popular method utilized to evaluate the ability of a substance to neutralize free radicals. It operates on the principle that a compound which provides a hydrogen atom to a radical is an antioxidant. This method assesses the scavenging ability of antioxidants towards the stable DPPH radical, which is a readily available organic nitrogen radical. Once an antioxidant donates a hydrogen atom to the DPPH radical, it becomes reduced, and the purple color of the radical fades to yellow. This reaction is stoichiometric, signifying that the amount of DPPH radical that is reduced is proportional to the concentration of antioxidant. By measuring the decrease in the absorption of the DPPH radical at 517 nm using a UV spectrometer, one can accurately and easily determine the antioxidant potential of a substance (Moon et al, 2009).

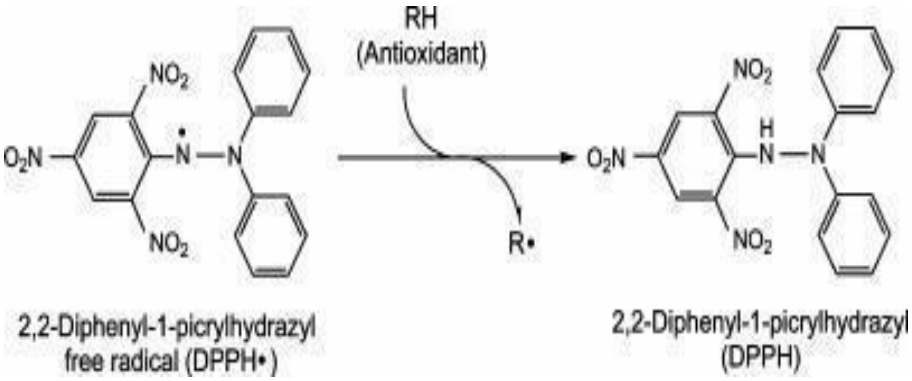


Figure.1.3: Mechanism of DPPH• free radical (Moon et al, 2009).

2.7.4 FRAP assay:

The Ferric Ion Reducing Antioxidant Power Assay (FRAP) is a commonly used technique for assessing the antioxidant capacity of biological and food samples. The FRAP assay measures the sample's ability to reduce Fe^3+ to Fe^2+ in the presence of a complexing agent, typically 2,4,6-tripyridyl-s-triazine (TPTZ), resulting in the formation of a blue Fe^2+-TPTZ complex. Antioxidants with reducing power are capable of reducing Fe^3+ to Fe^2+ and thereby increasing the absorbance at 593 nm, which can be measured with a spectrophotometer. This method is cost-effective, quick, and straightforward, and it does not require specialized equipment. In nutritional science, the FRAP assay is commonly used to assess the antioxidant capacity of various food samples and dietary supplements (Prior et al., 2005).

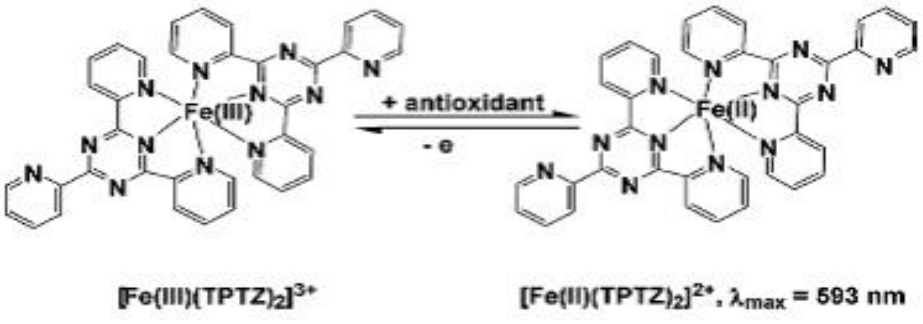


Figure 1.4: Reduction of yellow Fe^{3+} TPTZ complex (2,4,6-tri (2-pyridyl)-1,3,5-triazine) with antioxidants to the blue Fe^{2+} TPTZ complex by FRAP reagent (Prior et al, 2005).

2.8. Olive fruit fly infestation

The olive fruit fly is a major pest in Palestine that causes damage to olives and negatively impacts the quality of olive oil and table olive production. The fly and its larvae feed on the fruit, causing pulp damage and creating entry points for bacteria and fungi that can lead to fruit rotting and premature drop. Also, the damage of fruit pulp mix the olive oil with lipase enzyme which destroy the fatty acid Tri glycerol and elaborate fatty acids from triglyceride which increase Acidity of olive oil. The fly lays its eggs inside the olive fruit by puncturing it when an adult fly lands on it, initiating its life cycle (Ana Maria Gómez-Caravaca et al. 2008).

The olive fruit fly is a major pest that belongs to the subfamily Dacinae, causing significant damage to olive cultivation as its larvae feed on the fruit of olive trees (as shown in Figure 1.5). The life cycle of the fly begins with the adult fly landing on an olive and laying eggs inside the fruit's meat by puncturing it. Upon hatching, the larvae (as shown in Figure 1.6) feed on the pulp of the fruit, causing damage and providing entry points for secondary bacteria and fungi, ultimately leading to fruit rotting, beside mixing the lipase enzyme with olive oil which cause the dissociation of triglyceride and elaborate fatty acids and increase free fatty acids and oxidation in the olive oil. This can cause premature fruit drop and negatively affect the production of both table olives and olive oil (Ana Maria Gómez-Caravaca et al., 2008).

Fig1.5: The olive fruit fly (Bactrocera oleae)

Fig 1.6 : larvae of olive fly

An individual female olive fruit fly has the capacity to lay up to 250 eggs within olives. A complete life cycle takes approximately 35 days, and one generation can yield up to 250 flies. With up to four generations per season, a single female fly can give rise to over 3 million flies, resulting in significant damage to the olive crop for the entire season. The weather patterns greatly influence the population growth of the olive fruit fly, as hot and dry weather leads to a reduction in population, while moderate and humid weather causes an increase in population (Ben-Yosef et al., 2015).

The impact of olive fruit fly infestation on the quality of olive oil has been studied, and Gómez-Caravaca et al. (2008) investigated the effects of Bactrocera oleae infestation on the chemical parameters and phenolic profile of Italian virgin olive oils. Various olive oils were obtained from olives with different degrees of fly infestation. The study found that the degree of fly attack was positively correlated with free acidity and oxidized products, while the oxidative stability index and phenolic content were negatively related. However, it is essential to consider that multiple factors can influence the phenolic content of olive oil, and a direct relationship between the percentage of fly infestation and phenolic content may not always exist (Tamendjari and colleagues 2004; Lynda Amedjkouh et al. 2016).

Antonio Saltini (1989) suggested that some farmers prefer to use natural pest control methods to minimize infestations and accept a certain amount of fruit loss due to infestation, rather than using chemical pesticides.

The olive fruit fly is a significant pest in several Middle Eastern countries, and its impact on the crop can be severe, leading to losses of up to 60%.

With a long lifespan of several months, the olive fly is a highly prolific insect that can produce multiple generations annually and exclusively feeds on olives. Each female fly is capable of laying up to 200 eggs, with only one egg being deposited in each fruit. (Antonio Saltini, 1989).

Chapter 3. Methodology:

3.1 Materials :

The collected olive fruits were separated into two categories based on whether they were infested with olive fruit fly or not. The first group contained fruits that were completely infested, while the second group consisted of healthy fruits without any infestation. On October 25, 2021, samples of olive fruits were collected from two locations in Ramallah, specifically from orchards in Beit Rima and Mazaree Nouban villages. Fruits were divided into two groups based on their infestation status: infected (100%) and healthy (0%). About one kilogram of each sample was collected and further subdivided into three samples. The samples were processed separately under identical conditions to obtain virgin olive oils..

3.2 Extraction of olive oil

Abencor system (small scale olive mill4) was used to extract olive oil from the olive fruit samples. The % yield of olive oil obtained was in the range of 20-27%.

3.3 Examinations and analyzes to detect the impact of the olive fly on the quality of the oil

a- Acid Value

The acid value serves as a means to measure the quantity of free fatty acids in an oil or fat, denoted in milligrams of potassium hydroxide required to neutralize one gram of the substance. Its primary purpose is to determine the extent of spoilage that may have occurred due to the breakdown of oil glycerides, which results in the creation of free fatty acids. Typically, the acid value is presented as a percentage of the total free fatty acids estimated as oleic acid. (Adapted from Hamid, F., & Hamid, F. H. 2016).

b- Procedure:

7 grams of oil were added to a 250 ml clean, dry Erlenmeyer flask , . , In another clean and dry Erlenmeyer flask, 50 ml of 96% ethanol and 2 ml of phenolphthalein solution were combined. Then, 0.1 N aqueous NaOH solution was used to neutralize the solution by appear the faint pink hue. The oil was then mixed with the neutralized

ethanol, and the resulting mixture was vigorously stirred and boiled for two minutes on a hot plate. 0.1 N aqueous NaOH solution was then used to titrate the mixture until a light but stable pink color appeared and persisted for one minute.

c- **Peroxide value**

A 250 ml conical flask with a glass stopper was filled with a solution of 30 ml of glacial acetic acid-Chloroform (3:1 by volume) and swirled to completely dissolve about 5g of oil. The flask was then stopped, 1 ml of saturated potassium iodide solution was added, and it was allowed to stand in the dark for one minute while being periodically shaken. 30 ml of recently boiled , cooled water was then added to the mixture. The contents were vigorously shaken and titrated with 0.01 N sodium thiosulfate penta hydrate solution until the yellow color was almost completely gone. The titration was then restarted with vigorous shaking to release all the iodine from the chloroform layer until the blue color appeared. Subsequently, approximately 0.5 ml of starch solution was added, and the titration was resumed with vigorous shaking to release all iodine from the chloroform layer until the blue color had barely disappeared. Similarly, a blank determination was carried out in the same manner, but without the oil sample.

d- K_{232} and K_{270}

The presence of conjugated dienes and trienes is indicated by the wavelength range of 230–270 nm, which has a higher absorption level. These compounds are formed when unsaturated fatty acids undergo autoxidation, as well as from their hydroperoxide and fragmentation by-products.

0.25 grams of the substance were dissolved in 25 milliliters of cyclohexane to create a 1% solution of the oil. A UV spectrophotometer with a 1-centimeter path length was used to examine the solution, and the absorbance at 232 and 270 nanometers was measured.

3.4 Extraction of polyphenolic compounds and antioxidants from olive oil

To determine FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) , TPC (total phenolic content), TFC (total flavonoid content), and antioxidant activities of the oil samples, an extraction procedure was used. To ensure

accuracy, the extraction process was applied to multiple oil samples, and each sample underwent multiple analyses. The results were shown as means and their corresponding standard deviations.

The samples of olive oil underwent an extraction procedure to obtain the desired compounds. 20 grams of the sample and 20 mL of n-hexane were first mixed together in a separatory funnel. Then, to obtain the resulting methanolic extracts, 10 mL of an 80:20 v/v methanol-water mixture was added to the funnel three times. The extractions were washed with n-hexane (20 mL) to eliminate residual oil and stored in a refrigerator until analysis. Replicate analyses were conducted for TPC, TFC, FRAP, and DPPH, and the mean value and standard deviation were recorded.

3-5 Total Phenolic Contents (TPC)

The method described by Singleton and Rossi (1965) was used to calculate the total phenolic content (TPC). First, 1.8 mL of a 10-fold diluted Folin-Ciocalteu reagent was added to 100 mL of the extract sample, and the mixture was left to stand for 5 minutes. The mixture was then given 1.2 mL of a 7.5% NaHCO3 solution and was left for 60 minutes. A UV-Vis spectrophotometer was used to gauge the solution's absorbance at 765 nm in comparison to a water reagent blank. The instrument was calibrated using known concentrations of gallic acid in aqueous solutions ranging from 100 to 500 ppm. The gallic acid equivalents (GAE) per gram of sample used to express the TPC values is milligrams (mg). The findings were presented as mean values with corresponding standard deviations.

3-6 Total flavonoid content TFC

One of the most significant quality indices, total flavonoids content (TFC), measures the overall antioxidant activity of a substance.

The aluminum chloride method was used to analyze TFC (Zhishen et al., 1999). One milliliter (1 ml) of olive oil extract was added to a 10-milliliter volumetric flask along with four milliliters of distilled water, three milliliters of 5% NaNO2, and three milliliters of 10% AlCl3.6H2O. The mixture was left to stand at room temperature for 6 minutes. After adding two milliliters of 1 N NaOH, the solution was diluted with distilled water to make 10 milliliters. At 510 nm, the solution's absorbance was

immediately measured in comparison to a blank. Known catechin concentrations in aqueous solutions between 30 and 200 ppm were used for calibration curve.

3-7 Free radical scavenging activity using DPPH:

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical (Brand-Williams et al., 1995).

Materials used were: DPPH(2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl**),** Methnol (95%), Torolox, UV-Vis Spectrophotometer.

Procedure:

The antioxidant activity of the samples of olive oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. First, a methanol solution of DPPH was combined with each sample extract, and the mixture was left for 30 minutes until the absorbance reached a steady state. Then, by comparing the control absorbance to the absorbance of the sample extract, the percentage inhibition of DPPH by the sample extract was determined. By using various Trolox concentrations, a standard curve was created to calculate the antioxidant activity. The results were reported as mean values with their corresponding standard deviations and expressed as micromoles of Trolox per gram of sample**.**

3-8 Ferric reducing antioxidant power FRAP

It appears that the FRAP assay is being used to evaluate the antioxidant activity of the olive oil samples. This procedure involves combining the sample extract with a mixture of tripyridyltriazine (TPTZ) in acetate buffer, ferric chloride (FeCl3), and hydrochloric acid (HCl). The mixture is incubated for 30 minutes at 37°C, resulting in the reduction of ferric-tripyridyltriazine to the ferrous complex, which results in a blue color. After 4 minutes, the absorbance at 593 nm is measured and compared to a control. The ferrous ion concentrations in the aqueous solutions, which range from 2 to 5 mM, are known and used to create the calibration curve. Micromoles of Trolox per gram of sample are the units used to express the results. The FRAP assay measures antioxidants' capacity to convert ferric ions into ferrous ions, it is a widely used method for evaluating the antioxidant capacity of food samples.

3.9 Statistical analysis

SAS software (SAS Institute Inc., Cary, USA, Release 8.02, 2001), which was used for statistical analysis, was used to present the mean values and their corresponding standard deviations. The GLM procedure was used to perform an ANOVA on the means, with individual analyses carried out for each factor. T-tests were used to compare the infected and control samples, and multiple comparisons were made using the Bonferroni method with a 5% experiment-wise error rate. The relationships between specific quality indicators and other quality indices were also calculated using Pearson correlations. To ensure consistency with subsequent multiple regression analyses, the NOMISS option was used.

Chapter 4. Results and Discussions

4.1 Effect of olive infestation on the quality of olive oil

The aim of this study is to investigate the effect of olive fly infestation on the quality of Palestinian olive oil. Olive fly infestation can decrease oil yield by reducing the amount of oil produced in the olives. The larvae of the fly feed on the olive pulp, which can result in higher acidity and peroxide levels in the oil, potentially affecting its quality. In addition, the exit holes caused by the fly infestation can promote oxidation and hydrolytic degradation of the olive by exposing the pulp to microorganisms and oxygen.

Olive oil is a crucial component of the Mediterranean diet and is recognized for its potential health benefits, such as reducing the risk of cardiovascular diseases and colon cancer, and promoting overall health due to its antioxidants and high levels of oleic acid. However, various factors, such as cultivar type, fruit ripeness, and industrial extraction methods, can influence the quality and chemical composition of olive oil. Another significant factor that can impact olive oil quality is the level of infestation by the olive fruit fly, Bactrocera oleae.

The olive fruit fly is a destructive insect that can cause considerable harm to olive tree farming, resulting in economic losses by decreasing the quality and quantity of olive oil produced. The fly's larvae consume the fruit, causing damage to the pulp, increasing the vulnerability of the fruit to secondary microorganisms, and eventually resulting in fruit rotting and the deterioration of the oil. Previous studies have shown the negative effects of olive fruit fly infestation on the quality, bioactive compounds, and functional properties of olive oil. However, there has been a lack of research on how it affects antioxidant activity (Corrado G. et al. 2012; P. Vossen and A. Devarenne, 2006; Nardi F. et al. 2010; Gomez-Caravaca A.M. et al 2008; Gucci R. et al. 2012).

4.2 Quality parameters of the olive oil

The study aimed to investigate the impact of olive fly infestation on the quality of olive oil by analyzing parameters such as acidity, peroxide index, and UV absorbance values (K232 and K270). The findings indicated that with an increase in the degree of infestation, the quality of olive oil deteriorated, as indicated by the rise in acidity, peroxide index, and UV absorbance values. In particular, the acid values of olive oil from infected samples were 2.30±0.036 and 2.68±0.020 for Beit Rima and Noubani farmers, respectively, while uninfected samples had acid values of 0.75±0.015 and 0.76±0.015 from the same villages. This increase in acidity by three times led to the reclassification of olive oil from extra virgin (healthy oil) to virgin olive oil (unhealthy oil) based on acid values. Statistical analysis revealed a significant difference in acid values between affected and unaffected fruits (denoted as A and B in Figure 4.1), indicating that the acid values of olive oil extracted from affected fruits were significantly higher.

The study's findings show that olive fly infestation has adverse effects on olive oil quality, as indicated by significant increases in acidity and peroxide values, two critical parameters for evaluating olive oil quality. These high values imply that the oil is of lower quality and may have detrimental health consequences. This conclusion aligns with earlier research that has also reported the harmful influence of olive fly infestation on olive oil quality. The study emphasizes the need to control olive fly infestation to preserve olive oil quality and its health-promoting properties. (denoted as A and B in Figure 4.2).

To summarize, high levels of K232 and K270 values indicate that the olive oil has been oxidized due to the presence of the olive fly. This indicates that the oil has a shorter shelf life and a decrease in quality. The presence of free fatty acids in the oil due to the breakdown of the olive pulp by the larvae of the olive fly due to activation of the lipase enzyme, may also contribute to the increase in these values. The study concludes that the olive fly attack has a significant negative impact on the quality of olive oil, as demonstrated by increased acidity, peroxide index, and K232 and K270 values (denoted as A and B in the figure).

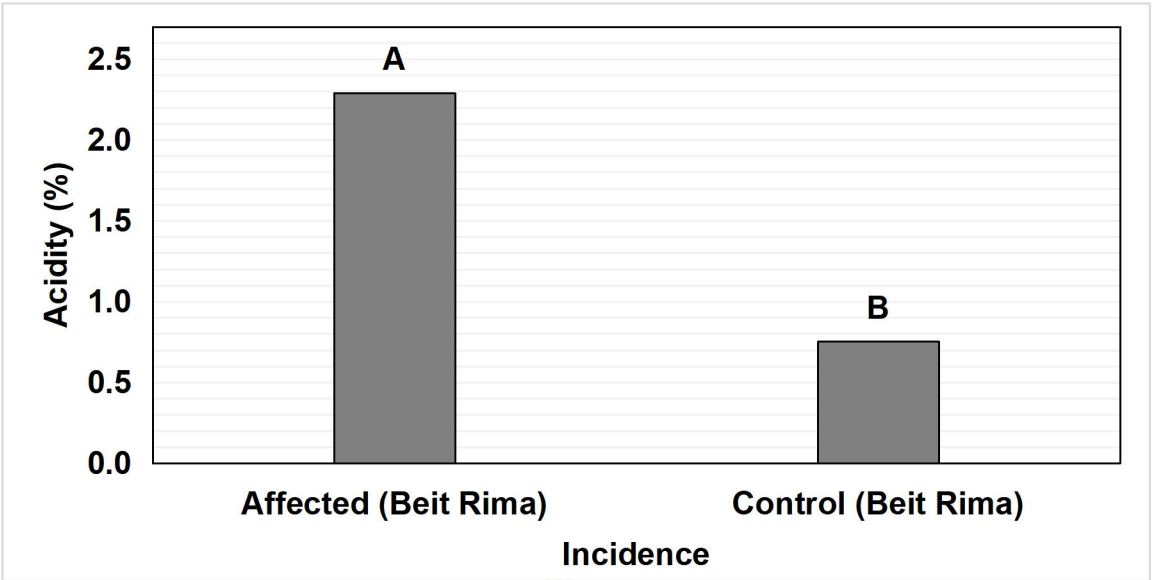
The study's results align with international standards, which classify olive oil derived from healthy fruit as extra virgin, while that derived from attacked fruit is classified as

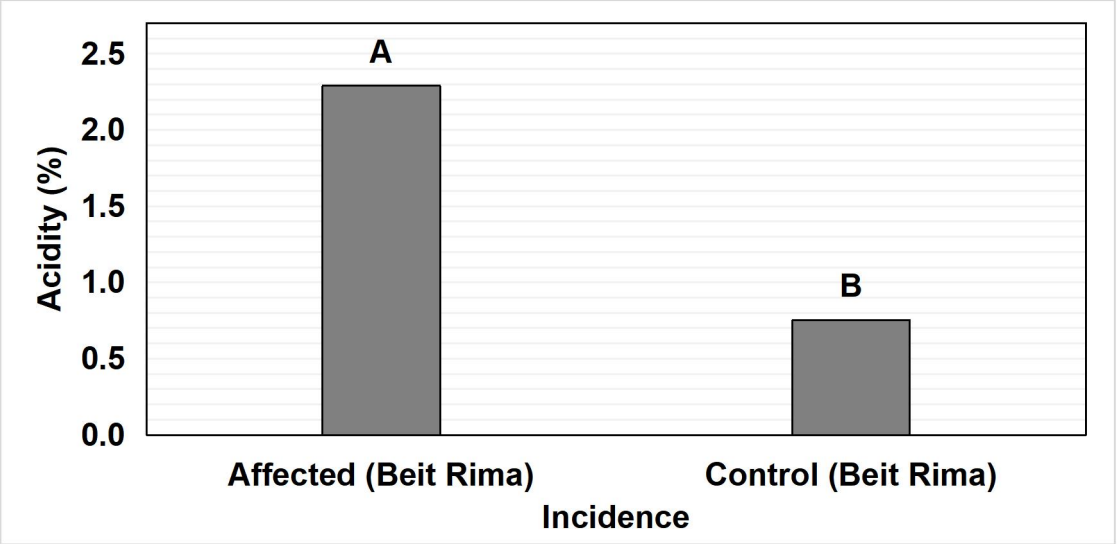
virgin due to degradation caused by hydrolytic and oxidative processes. These processes occur when exit-holes expose the olive pulp to environmental factors, leading to the growth of bacteria, yeast, and fungi that damage the pulp further. Similar findings have been reported by other researchers, including J.A. Pereira et al. (2004), F. Mraicha et al. (2010), A. Tamendjari et al. (2004), and H. Topuz and E. Durmusoglu (2008).

Table 4.1: The quality parameters, including acid value, peroxide value, K232, and K270, of olive oil samples obtained from olives infected with olive fly were compared to those obtained from unaffected olives to determine the impact of the fly infestation on the quality of the resulting oil..

Sample	Acid value (oleic acid)	Peroxide value (meq O2 /Kg)	K232	K270
100% affected from Beit Rima	2.30±0.036	7.50±0.15	1.91±0.021	0.31±0.015
100% affected from Noubani farmers	2.68 ±0.020	9.60±0.15	2.44±0.015	0.33±0.017
Control (unaffected from Beit Rima)	0.75 ±0.015	2.42±0.015	1.48±0.020	0.21±0.010
Control (unaffected from Noubani farmers)	0.76±0.015	3.64±0.050	1.58±0.015	0.23±0.057

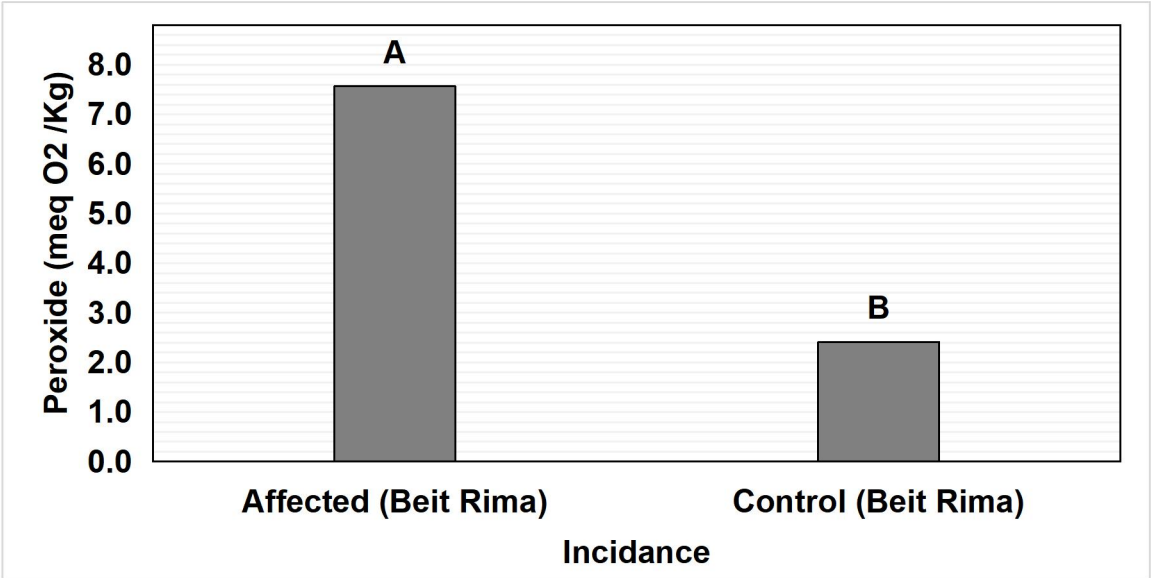
* values are reported as Average ± SD (n = 3 samples).





(b)

Figure 4.1: Acid value of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers. (A and B refers to statistical difference where A is significantly higher than B).



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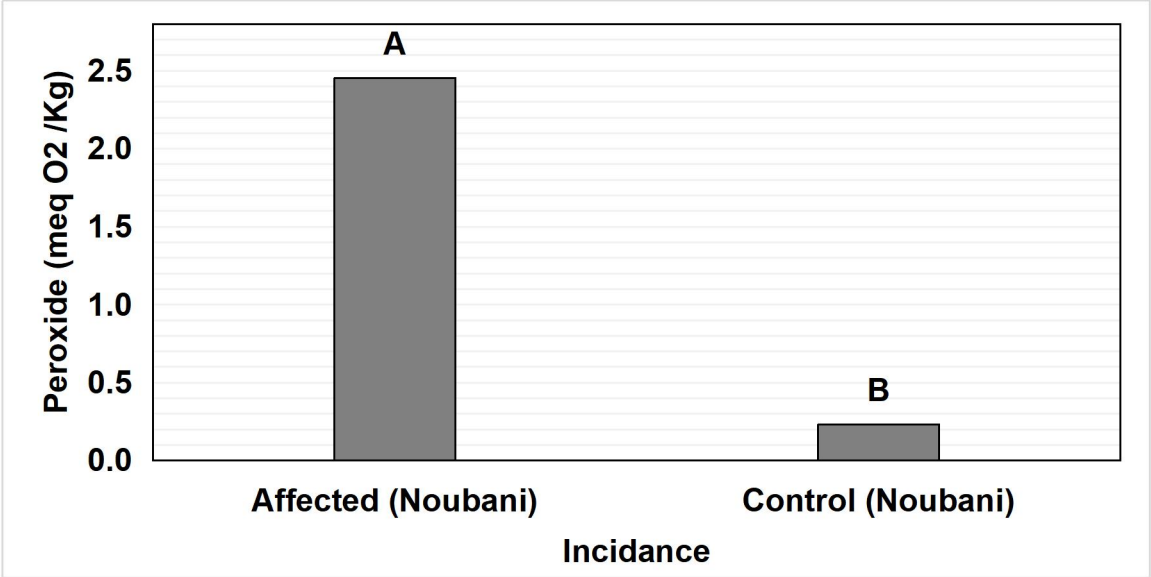

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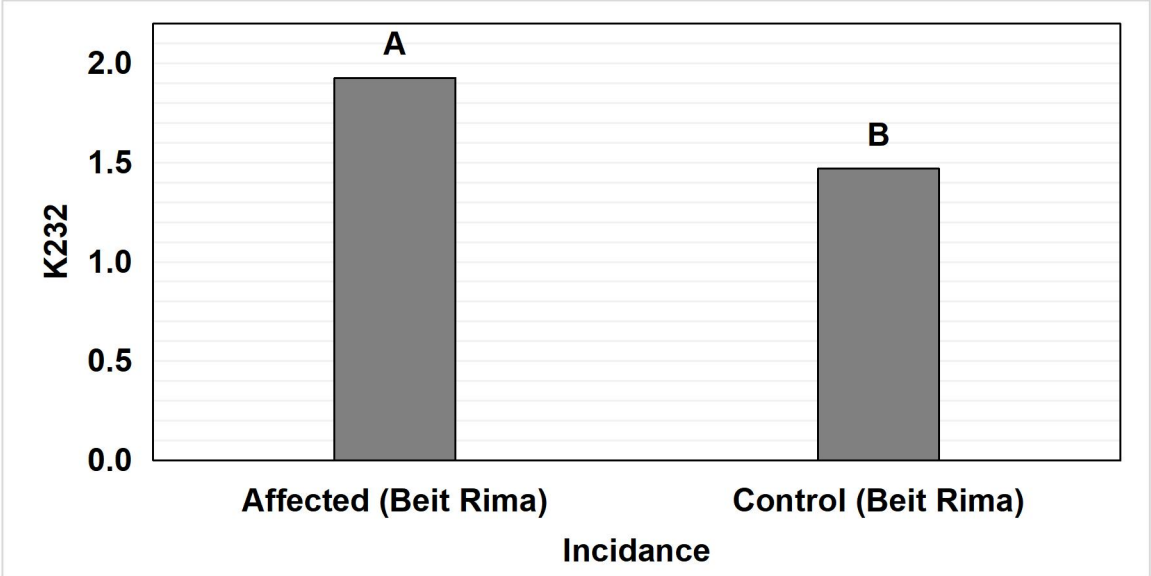
Figure 4.2: Peroxide value of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers.



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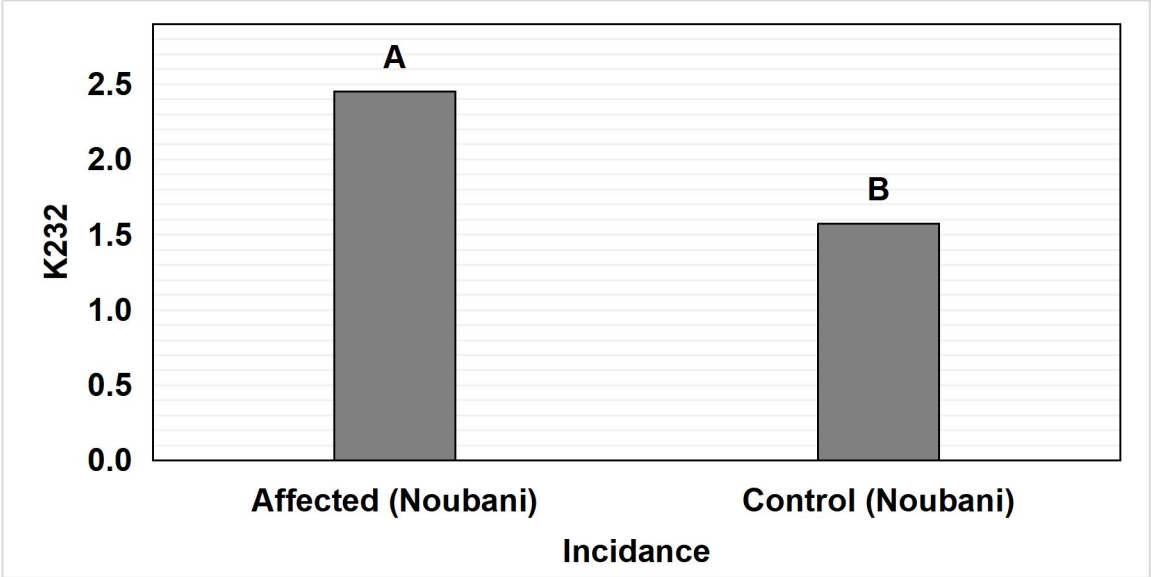
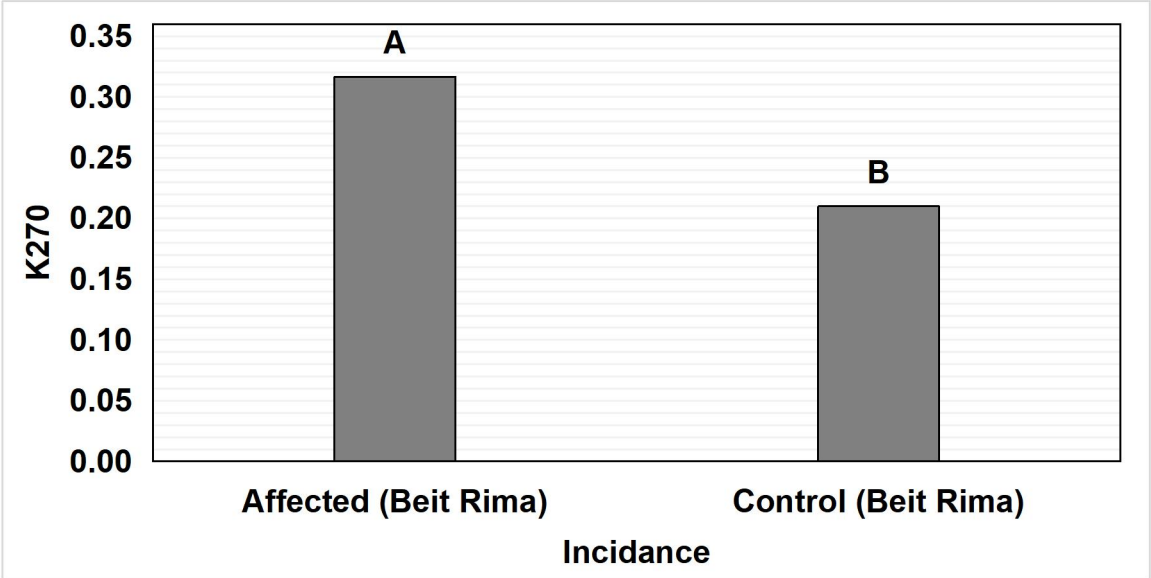


(b)

Figure 4.3: K232 values of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers.



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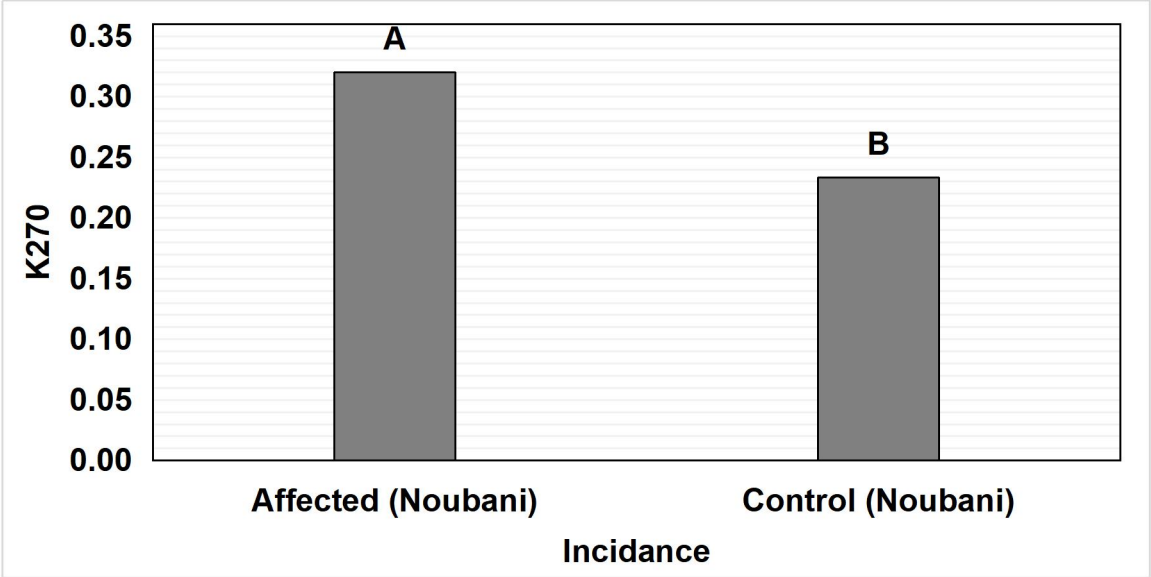


(b)

Figure 4.4: K270 values of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers.

4.3 Total phenolic and flavonoids contents

The study utilized a spectrophotometric method at 765 nm to measure the total phenolic contents of the olive oil samples. A calibration curve was generated using gallic acid within the concentration range of 100-500ppm, and the curve was found to have good linearity with an R2 value of 0.9988. The total phenolic content of the olive oil extracts in the study was then calculated using this calibration curve.

Table 4.2: Absorbance of different concentrations of Gallic acid

Concentration of Gallic acid (ppm)	Absorbance at λ= (765 nm)
100	0.13
200	0.43
350	0.83
450	1.07
500	1.20

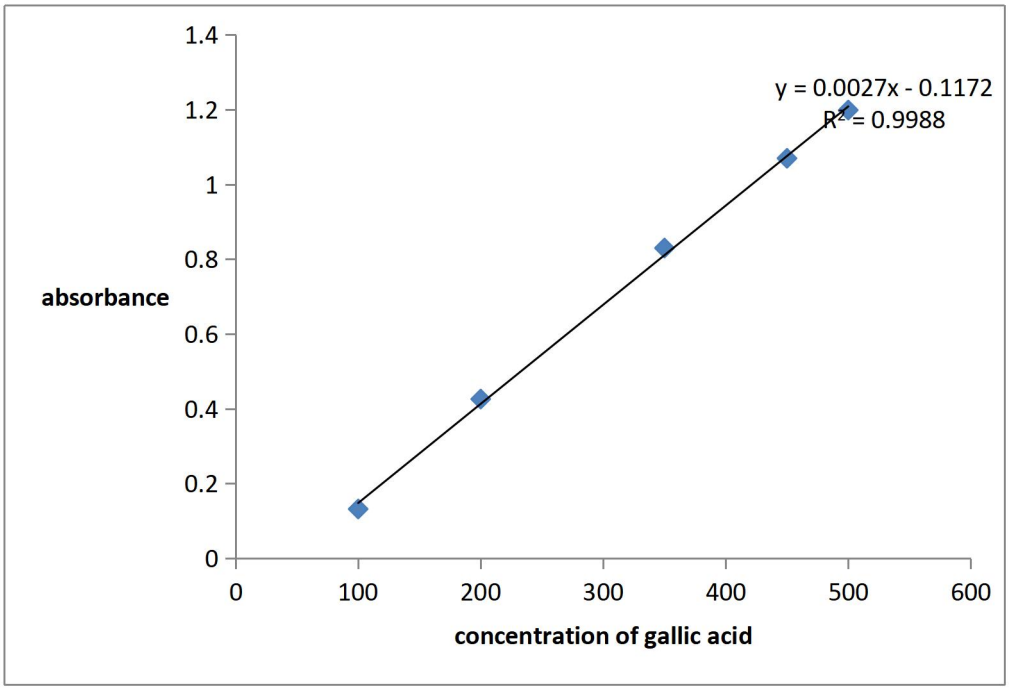


Figure 4.5: Calibration curve for total phenols content.

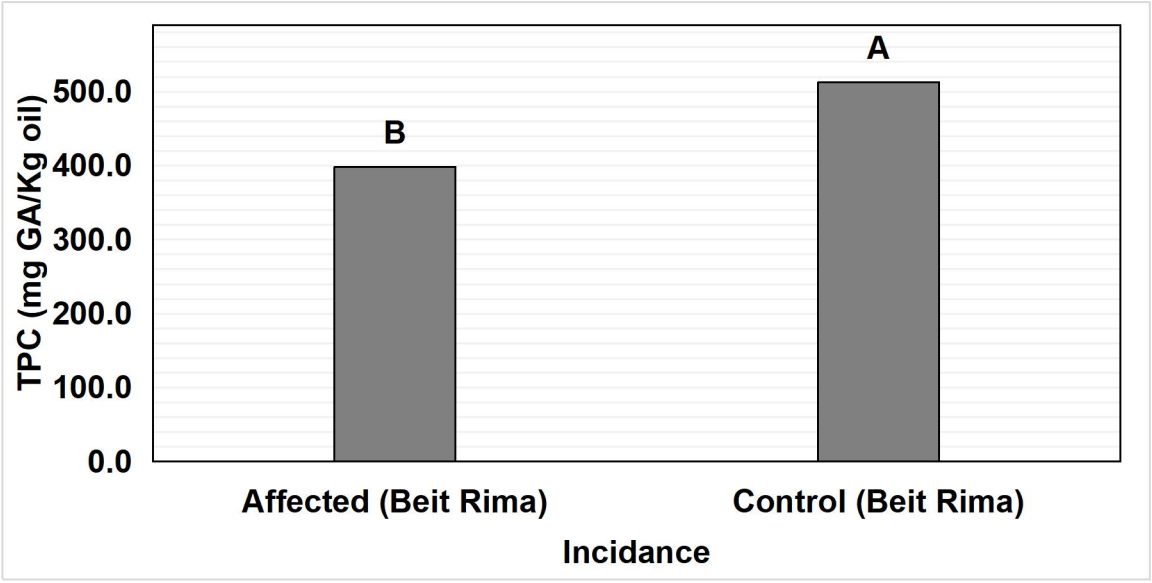
Table 4.3 presents the results of the analysis of total phenolic and flavonoid contents of olive oils obtained from different degrees of olive fly infestation. The olive oil samples extracted from olives that were 100% attacked by the fly exhibited the lowest phenolic content, with values of 397±3.0 and 410±2.0 for oils from Beit Rima and Noubani farmers, respectively. On the other hand, the phenolic content of olive oils obtained from uninfected olives from the same farmers was higher, with values of 514±2.5 and 493±3.2. The loss of total phenolic compounds in the olive oils extracted from 100% infested olives was 23% and 17% for samples obtained from Beit Rima and Noubani farmers, respectively. The statistical analysis revealed significant differences in the total phenolic contents of olive oil extracted from affected and unaffected fruits, with the former having significantly lower phenolic content values (indicated as A and B in Figure 4.6).

Table 4 3: Total phenols (mg GAE per kg oil), total flavonoids (mg QE per kg oil) of olive oil from fruits infected with olive fly compared to olive oil from unaffected olive fruits.

Sample	TPC (mg GA/Kg oil)	TFC (mg catechin/Kg oil)
100% affected from Beit Rima	397±3.0	101±1.5
100% affected from Noubani farmers	410±2.0	111±1.5
Control (unaffected from Beit Rima)	514±2.5	158±2.6
Control (unaffected from Noubani farmers)	493±3.2	173±2.0

* values are reported as Average ± SD (n = 3 samples).

Previous research by Tamendjari et al. (2009) investigated the effect of olive fly infestation on the total phenolic content in olives, and their findings also revealed a significant reduction in phenolic content, particularly in more mature olives. The decline in phenolic content in the attacked samples is believed to be attributed to the increased activity of polyphenoloxidase, which is facilitated by the entry of oxygen through the exit-holes created by fly larvae, as noted by Pereira et al. (2004). Other studies, such as Tamendjari et al. (2004), have also reported similar results on the decrease in phenolic content due to olive fly infestation.



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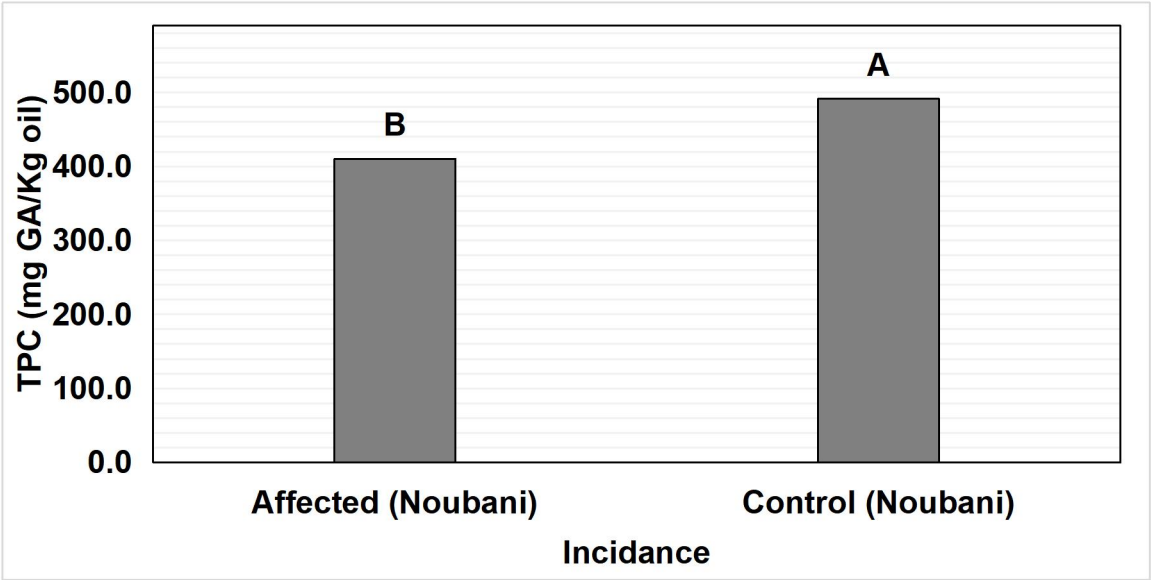


(b)

Figure 4.6: Total phenoloc content of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers.

4.4 Total flavonoids content

The researchers employed a spectrophotometric method at 510 nm to assess the total flavonoid content of the olive oil samples. They utilized catechin as the standard to generate a calibration curve within a range of 50-100 ppm, and the outcomes are recorded in Table 4.4. The calibration curve demonstrated good linearity, with an R2 value of 0.977, as depicted in Figure 4.7. The researchers subsequently utilized the calibration curve to calculate the total flavonoid content of the olive oil extracts they examined in their study.

Table 4.4: Absorbance for different concentrations of Catechin.

Concentration of catechin (ppm)	Absorbance λ= (510 nm
50	0.255
60	0.282
75	0.353
86	0.396
100	0.496

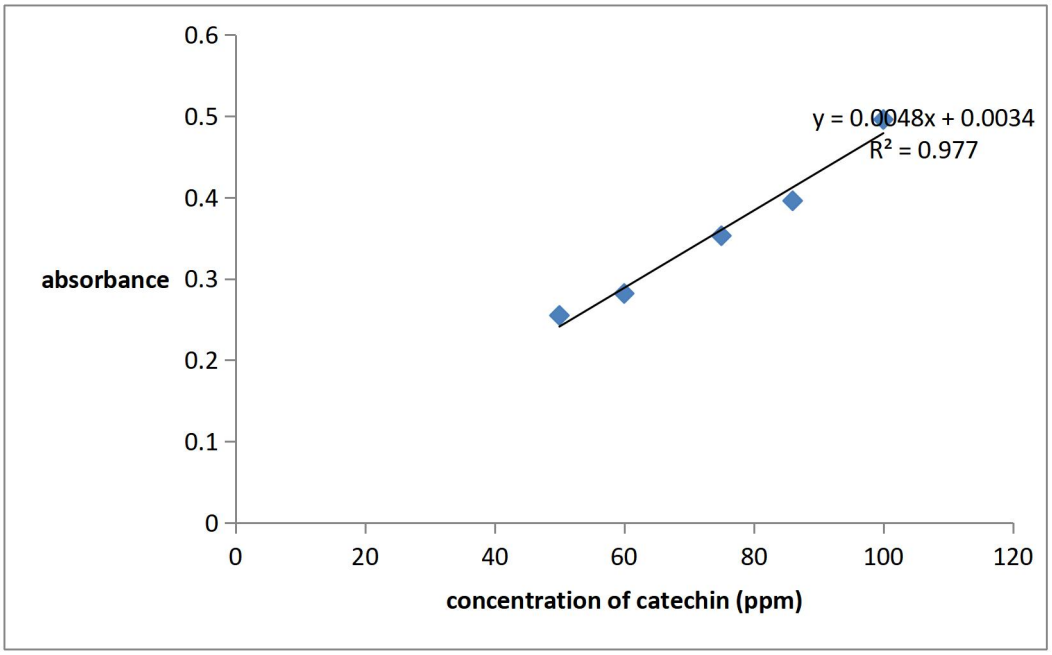
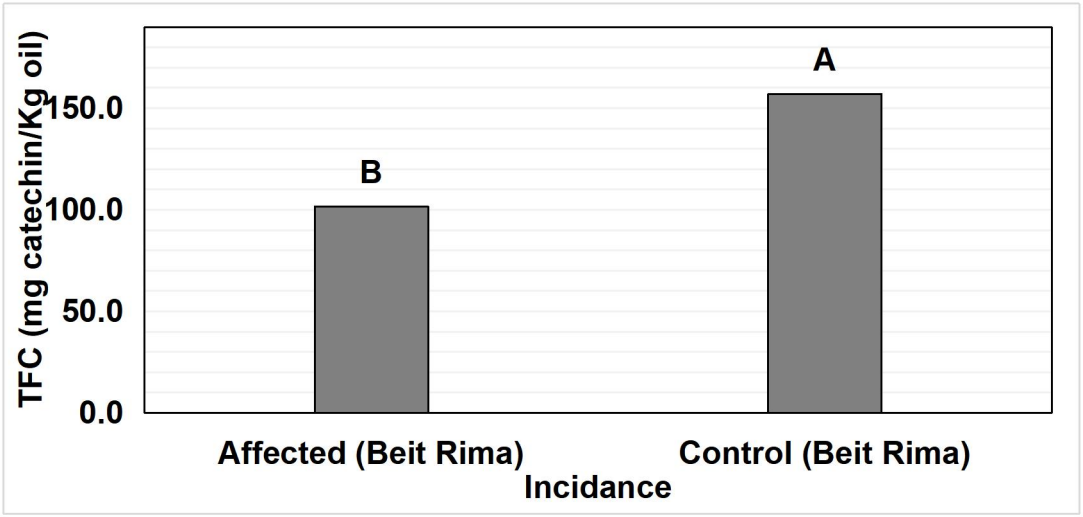


Figure 4.7: Calibration curve for total flavonoid content

Table 4.3 shows the total flavonoid contents of olive oil samples with different levels of olive fly attacks. The findings indicate a decrease in the total flavonoid content of olive oil samples obtained from attacked olives compared to healthy ones. For instance, in the case of olive oil samples from Beit Rima, the TFC was 158±2.6 and 101±1.5 for healthy and attacked olives, respectively. Similarly, for olive oil samples from Noubani farmers, the TFC for healthy and attacked olives were 173±2.0 and 111±1.5, respectively. The reduction in total flavonoid content in olive oils extracted from 100% attacked olives was 37% and 36% for samples from Beit Rima and Noubani farmers, respectively. Statistical analysis was conducted to determine significant differences in the total flavonoid content of olive oil samples from affected and unaffected fruits. The results in Figure 4.8 indicate that the total flavonoid content of olive oil from attacked fruits was significantly lower than that from healthy fruits (denoted as A and B in the figure).

Mraicha et al. (2010) carried out research which demonstrated a considerable decrease in the flavonoid content of olive oil obtained from olives that were fully attacked by the olive fly. The reduction was approximately 66% when compared to samples of healthy fruit. This underscores the importance of flavonoids, which belong to the subclass of phenolic compounds, in olive oil.

The decline in phenolic compounds as a result of olive fly infestation has a significant impact on the desirable taste qualities of olive oil, particularly its bitterness and pungency. Research conducted by Beltran et al. (2005) suggests that bitterness in virgin olive oil is largely associated with the total phenol content. Similarly, Morello et al. (2006) propose that the total phenol content is a more accurate indicator of the bitterness index, possibly due to the diverse range of phenolic compounds that contribute to the bitterness of virgin olive oil.



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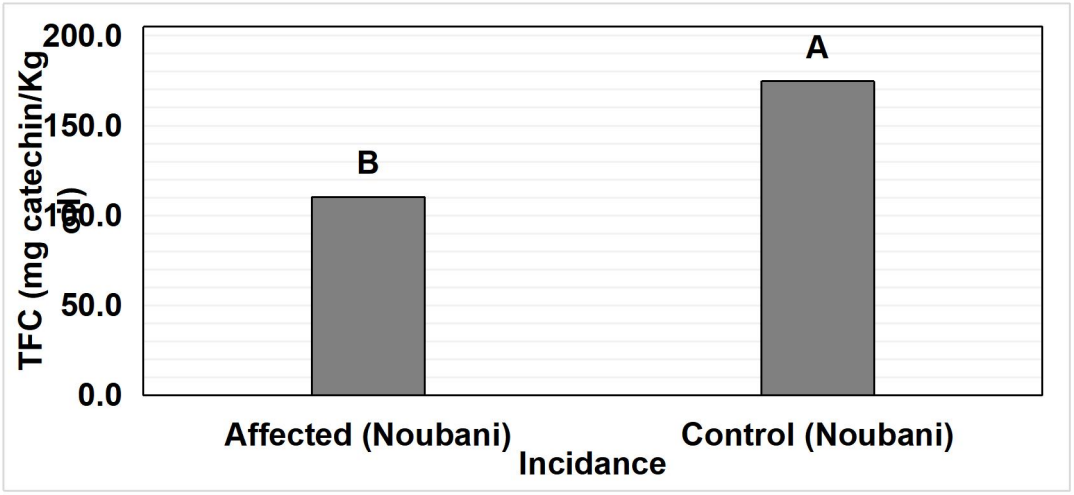


(b)

Figure 4.8: Total flavonoids content of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers

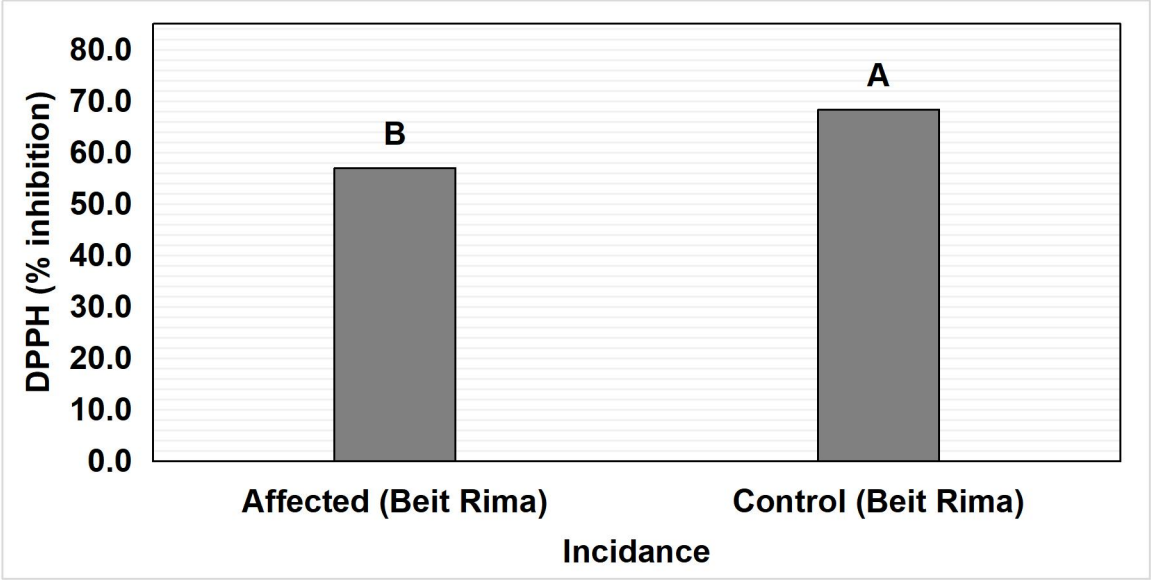
The figure displayed in Figure 4.8 represents the total flavonoid content of olive oil samples obtained from olives that were attacked by olive fly in comparison to the total flavonoid content of olive oil obtained from healthy olives. The data is presented separately for olive fruits collected from Beit Rima and Noubani farmers.

4.5 DPPH radical-scavenging activity

The DPPH method is commonly utilized to assess the antioxidant potential of various substances by evaluating their ability to eliminate free radicals or donate hydrogen atoms. The findings presented in Table 4.6 suggest that the level of olive fly infestation can affect the behavior of DPPH. A decline of approximately 20% was noted in the percentage of inhibition of DPPH in olive oil extracts from infested olive fruits when compared to those from healthy samples. For instance, in Beit Rima, the percentage of inhibition of DPPH was 56.3±1.0 and 67.7±1.52 for oil samples from infested and healthy olive fruits, respectively. In the Noubani farmers village, the percentage of inhibition of DPPH was 59.0±1.5 for unhealthy samples and 72.0±1.52 for healthy samples. This decrease in DPPH values of infested samples is thought to be due to the loss of polyphenols and flavonoids, as previously demonstrated in Tamendjari et al.'s (2004b) research. Additionally, the statistical analysis showed significant differences between the DPPH values of olive oil from infested and healthy fruits, as represented by A and B in Figure 4.9.

The DPPH radical scavenging activity is a widely used method to assess the antioxidant potential of foods and compounds by measuring their ability to act as free radical scavengers or hydrogen donors. Table 4.6 shows that the extent of B. oleae infestation can affect the DPPH behavior, as a decrease of approximately 20% was observed in the percentage of inhibition in olive oil extracts from infested olives compared to healthy ones. For instance, the percentage of inhibition of DPPH was 56.3±1.0 and 67.7±1.52 for oil samples from infested and healthy olives in Beit Rima, respectively. Similarly, the percentage of inhibition of DPPH was found to be 59.0±1.5 and 72.0±1.52 for unhealthy and healthy samples obtained from Noubani farmers village, respectively. The reduced DPPH values of the infested samples can be attributed to the loss of polyphenols and flavonoids resulting from B. oleae infestation, as previously reported by various researchers. The IC50 values for olive oil extracts from infested olives were 1.7 times higher than those from uninfected

olive fruits, indicating a loss of activity. This finding is consistent with previous studies that have reported a decline in antioxidant activity and an increase in IC50 values beyond 15% fruit infestation.



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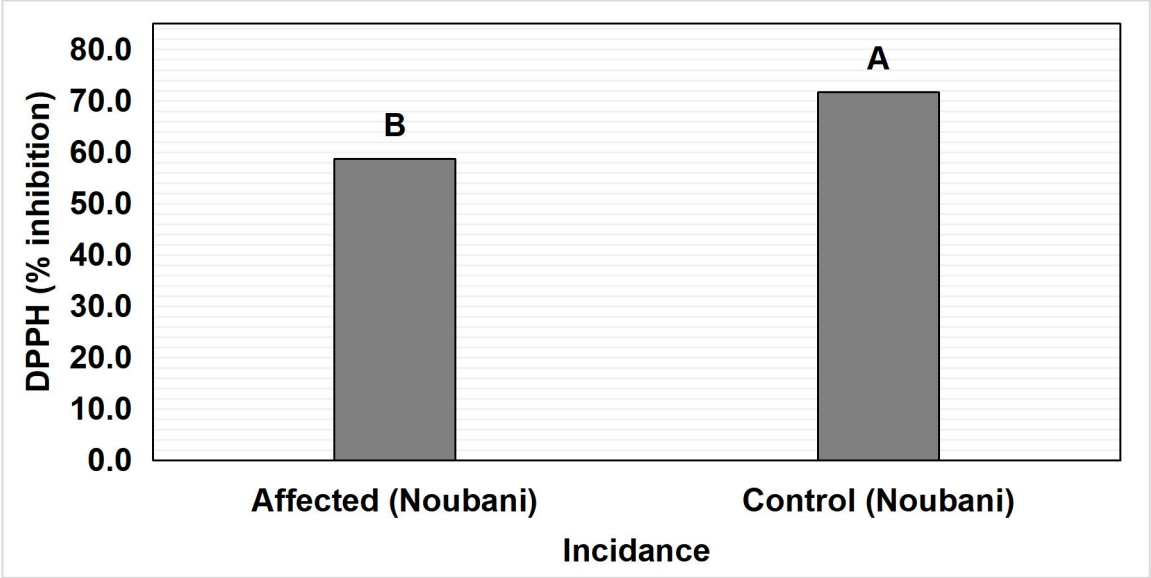

(b)

Figure 4.9: DPPH of olive oil samples from olive fruits infected with olive fly compared to olive oil from unaffected olive fruits (a): fruits collected from Beit Rima and (b) from Noubani farmers.

Figure 4.9 illustrates the DPPH values of olive oil samples extracted from olive fruits affected by B. oleae compared to those obtained from unaffected olives. Panel (a)

shows results obtained from fruits collected from Beit Rima, while panel (b) displays results from Noubani farmers.

4.6 Correlation of the olive fly attack with oil quality parameters

Table 4.7 presents the Pearson correlation coefficients for several parameters that are used to assess the quality of olive oil. These parameters include acidity, peroxide value, K232, K270, total phenolic content, total flavonoid content, as well as antioxidant activities measured using FRAP and DPPH assays. The correlation coefficients are presented for both infected samples (above the diagonal) and control samples (below the diagonal) from the two locations studied.

Table 4.7 presents the Pearson correlation coefficients between various parameters used to determine the quality of olive oil, including acidity, peroxide value, K232, K270, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities measured by FRAP and DPPH assays, for both control and infected samples from both sites. The results indicate that acidity was negatively correlated with peroxide value in both control and infected samples, but positively correlated with K232 and K232 in infected samples only. In control samples, acidity was slightly negatively correlated with TPC, while in infected samples, it was highly positively correlated with TPC. The correlation between acidity and antioxidant activities was not significant in control samples, but highly positively correlated with FRAP in infected samples. Peroxide value was significantly and negatively correlated with K232, K270, TFC, FRAP, and DPPH in control samples, while in infected samples, it was negatively correlated with all quality parameters except K270. The extinction coefficient K232 was positively correlated with all quality parameters in control samples, while in infected samples, the correlation was significant and positive in all parameters except K270. TPC was negatively and significantly correlated with TFC, FRAP, and DPPH in control samples, while in infected samples, it was positively correlated with TFC and FRAP only. TFC was positively correlated with both FRAP and DPPH in both control and infected samples, while FRAP was positively correlated with DPPH in control samples only.

Table 4.5 presents the Pearson correlation coefficient values for parameters that determine olive oil quality and its antioxidant activity, including acidity, peroxide value, K232, K270, total phenolic content (TPC), total flavonoid content (TFC), as well as FRAP and DPPH assays. The correlation coefficient values are presented separately for control samples and infected samples from both Beit Rima and Noubani farmers village. Values below the diagonal correspond to control samples, while values above the diagonal correspond to infected samples.

Variables	AC	PV	K232	K270	TPC	TFC	FRAP	DPPH
AC	---	-0.63*	0.48	0.28	-0.69*	0.72*	0.59	0.35
PV	-0.99***	---	-0.96***	-0.86*	0.98***	-0.98***	-0.92**	-0.80*
K232	0.98***	-0.99***	---	0.87**	-0.89**	0.94**	0.96**	0.75*
K270	0.17	-0.14	0.11	---	-0.85**	0.83**	0.70*	0.65
TPC	0.94**	-0.95**	0.95**	0.26	---	-0.95***	-0.82**	-0.76*
TFC	0.95**	-0.96***	0.96**	0.27	0.92**	---	0.94***	0.71*
FRAP	0.99***	-0.97***	0.96**	0.198	0.90**	0.90**	---	0.73*
DPPH	0.56	-0.62*	0.63*	0.17	0.55	0.77*	0.45	---

*indicate significant at P<0.05, **indicate significant at p<0.01, ***indicate significant at P<0.001, n=6

Chapter 5. conclusion

A significant pest in olive groves that is known to harm olive fruits is the olive fly, Bactrocera oleae. In olive-producing regions, B. oleae infestation of olives is a frequent issue that reduces yield and lowers the quality of the olive oil produced. Acidity, peroxide value, K232, K270, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity as determined by FRAP and DPPH assays are some of the factors that affect the quality of olive oil.

According to studies, the presence of B. oleae in olive fruits has a negative impact on the oil's quality and lowers its antioxidant activity. Olive oil's lower concentration of phenolic compounds has been linked to the loss of antioxidant activity brought on by infestation. Olive oil's antioxidant activity is known to be significantly influenced by the amount of phenolic compounds present, and it has been noted that antioxidant activity declines above a certain level of fruit infestation. As a matter of fact, it was discovered that the IC50 values for olive oil extracts from attacked olives were 1.7 times higher than those from unaffected olive fruits, indicating a decline in activity.

The study also found that the degree of B. oleae infestation was correlated with changes in various quality parameters of olive oil. Acidity was negatively and significantly correlated with peroxide value in both control and infected samples. However, acidity was not correlated with K232 and K270 in control samples, whereas it was positively correlated with K232 and not correlated with K270 in infected samples. The correlation between acidity and both antioxidant activities (FRAP and DPPH) was not significant in control samples, but highly and positively correlated with FRAP only in infected samples.

Peroxide value was significantly and negatively correlated with K232, K270, TFC, FRAP, and DPPH in control samples and highly positively correlated with TPC. In infected samples, peroxide value was negatively and significantly correlated with all quality parameters except K270. The extinction coefficient 232 was positively correlated with all quality parameters in control samples but negatively correlated with TPC. In infected samples, the correlation was significant and positive in all parameters except K270. The extinction coefficient K270 was positively correlated

with TPC and FRAP and negatively correlated with TPC in control samples but not correlated with any of the mentioned parameters in infected samples.

In conclusion, B. oleae infestation of olives has a detrimental effect on the antioxidant activity and quality of olive oil. The observed decline in olive oil's antioxidant activity may be caused by the loss of polyphenols and flavonoids brought on by B. oleae. To preserve the quality and health benefits of olive oil, it is crucial to control the olive fly infestation.

6. The References

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الملخص باللغة العربية

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الهدف من هذه الدراسة هو تقييم تأثير الإصابة بذباب ثمار الزيتون على معايير جودة زيت الزيتون المختلفة (النشاط المضاد للأكسدة ، محتوى الفينولات الكلي ، محتوى الفلافونويد الكلي ، القيمة الحمضية ، قيمة البيروكسيد ، K232 ، و .(K272 تميزت الثمار ذات الإصابة النشطة بوجود ثقوب يصنعها حشرة ذباب الزيتون. أظهرت نتائج هذه الدراسة زيادة في القيم الحمضية وقيم البيروكسيد وقيم ال K232)و (K272 لزيت الزيتون من عينات ثمار الزيتون المهاجم بذبابة الزيتون مقارنة بعينات الزيت الصحي (من ثمار الزيتون غير المهاجمة بالذبابة). وهذا يعني تقليل جودة زيت الزيتون الناتج عن الإصابة بذبابة الزيتون. يمكن أن يعزى ذلك إلى تسريع الأكسدة والتحلل المائي ، والمفضل بسبب وجود فتحات خروج تعرض لب الزيتون لتأثير الكائنات الحية الدقيقة والأكسجين. أظهرت النتائج أيضًا انخفاض محتوى الفينول والفلافونويد الكلي لزيوت الزيتون من ثمار الزيتون المصابة مقارنة مع زيت الزيتون من الثمار المصابة مما يشير أيضًا إلى انخفاض جودة الزيت لأن هذه المركبات البوليفينولية تعمل كمضادات للأكسدة وتلعب دورًا مهمًا في استقرار ومدة الصلاحية لزيت الزيتون. وبالمثل ، هناك انخفاض في أنشطة مضادات الأكسدة (التي تنعكس في فحوصات FRAP و (DPPH لزيوت الزيتون من الثمار المصابة والتي تشير أيضًا إلى تراجع جودة زيت الزيتون.