Natural Induction of Potato Crop Resistance by Plant Essential Oil Elicitors to Control Green Peach Aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

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تحفيز المقاومة الطبيعية لمحصول البطاطا باستخدام الزيوت العطرية النباتية كمحفزات لمكافحة مَن الدراق الذهبي *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

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This thesis/dissertation (Natural Induction of Potato Crop Resistance by Plant Essential Oil Elicitors to Control Green Peach Aphid (*Myzus persicae* (Sulzer) (Homoptera: Aphididae) was successfully defended and approved on 10th June 2020.

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التاريخ.................

Natural Induction of Potato Crop Resistance by Plant Essential Oil Elicitors to Control Green Peach Aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

Dedication

To my life and the source of my strength, my mother

To the memory of my father,

To my brothers, and my sisters,

To my supervisor Dr. Rana Samara

To my friends.

Acknowledgment

First of all, Praise to You, Allah, and thank you for helping me to accomplish this project.

I would like to thank my supervisor, Dr. Rana Samara, for her efforts, patience, and guidance to make this project as the best as it can be. Also, I am grateful to everyone who helped me.

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Abbreviation	Description
%	Percent
ºC	Celsius degree
µL	Microliter
µM	Micromolar
Cm	Centimeters
DW	Distilled water
g	Gram
h	Hour/ hours
H_2O_2	Hydrogen peroxidase
IPM	Integrated pest management
ISR	Induce systemic resistance
SAR	Systemic acquired resistance
JA	Jasmonic acid
SA	Salicylic acid

PR	Pathogen Related
K_3PO_4	Potassium phosphate
M	Molar
Mg	Milligram
Min	Minute/ Minutes
nm	Nanometer
NPK	Nitrogen: phosphorus: potassium
EPG	Electrical Penetration Graph
PLRV	Potato Leaf Roll Virus
PVY	Potato Virus Y
PVA	Potato Virus A
GPA	Green Peach Aphids
HR	hypersensitive response
PCD	programmed cell death
SE	Sieve Element
NP	Non Probing

E1	Salivation
E2	Phloem Sap Removal
C	Pathway
G	Xylem feeding
F	Difficulty Feeding Delaying
PD	Potential drop
LC_{50}	Lethal concentration
Df	Degree of freedom
NO	Number
rpm	Round per minute
UV	Ultraviolet
Ppm	Part per million
W.V	Weight per volume
DC	Direct current
Ω	Ohm a unit of electrical resistance
Std	Standard deviation

ROS	Reactive Oxygen Species
PGPR	Plant growth-promoting Rhizobacteria
Ph	Acidity or basicity
EO	Essential oils
POX	Guaiacol-Peroxidase
PPO	Polyphenol oxidase
SAS	Statistical analysis software
Sec	Second/ Seconds
PTUK	Palestine Technical University - Kadoorie
PVP	Polyvinylpyrrolidone

Natural Induction of Potato Crop Resistance by Plant Essential Oil Elicitors to Control Green Peach Aphid *Myzus persica* (Sulzer) (Homoptera: Aphididae)

By: Ru`ya Abu-Alfayah

Supervised by: Dr. Rana Samara

Abstract

Potato, *Solanum tuberosum* L., (Solanaceae) is one of the essential basic food crops worldwide. It has been attacked by many insect pests, such as aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). This research amid to use plant essential oil elicitors to improve the controlling method of the green peach aphid *M. persica*e.

In this study, different essential oils (EO) from medicinal plants were tested against *M. persicae*. Assessment of their potential role in inducing plant resistance pathways on potato ‘Sponta” cultivar was studied. Measuring two common enzymes indicators such as guaiacol Peroxidase (POX) and Polyphenol oxidase (PPO) expressed in plants post-physical or chemical injury. The Electrical Penetration Graph (EPG) was also used to monitor changes in aphid behaviors. The impact of the EO on aphid feeding behavior

was monitored and recorded for 8 hrs post-treatment. The effect of the medicinal plants' oil on the aphid mortality was carried out and measured in vitro

Results showed that 60% of the essential oil extracts have insecticidal activity against M.*persica*. Results also revealed that some EO have significantly increased the level of POX or PPO, and insect behavior compared to the control. Mustard, Sage, Jojoba, Eucalyptus, Bitter cucumber, Camphor, and Rosemary oil have natural induction in potato. Camphor, Sage, Pomegranate, and Bittercucumber caused a disturbance in GPA settling behavior and prolong searching for a feeding site. Mustard, Eucalyptus, and Sage resulted in delaying drop potential associated with non-persistent and semi-persistent. Lavender, Eucalyptus, and Mustard caused a delay in sieve element behavior associated with persistent virus transmission.

1 Introduction

1.1 General introduction

Potato *Solanum tuberosum L*., (Solanaceae: Solanales) with an estimated 1400 species (Wahlert et al., 2014) is considered as one of the main food sources after rice and wheat in many countries (Devaux et al., 2014). Potatoes products could be used as fresh, frozen, or dehydrated, but they are mainly used in food processing such as potato chips, French fries, and other processed products (Zhang et al., 2017). Potatoes contain a high percentage of carbohydrate, starch, and the basic nutrients such as protein, fibers, and vitamins especially vitamin C, B6, and B1, they are a very rich source of potassium, phosphorus, calcium, magnesium, zinc, and micronutrient (Storey, 2007). Potato cultivated area in the West Bank has reached 21177 (dounm) equivalent to a ratio of 4.3% of the total area used for field crop cultivation. The total production of potato was 70000 tons/year, 38% of the total field crop production in Palestine, (PCBS 2007-2008). In 2015, the area planted with potatoes was equal to 10,835 (dounm)equivalent to a ratio of 3.2% of the total area used for field crop cultivation. The total production of potato was 37,552 tons/year, 11% of the total field crop production. Tubas government was the largest area cultivated with potato (41%), followed by

Nables government (30%).The total production portion was in Tubas government (43%) and in Jenen government (27%), PCBS(2013-2015).

Potato crop has been attacked by many insect pests, which caused damage by either direct feeding on the tubers or the vegetative part, or indirectly by transmitting pathogens. Green peach aphids (GPA) *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is the most economically important insect pests on Potato, it causes damage primarily through their direct feeding on the plant phloem, and transmission of over than 200 viral diseases such as Potato Leaf Roll Virus (PLRV), Potato Virus Y (PVY), and Potato Virus A (PVA) (Westwood & Stevens, 2010). Non-persistent viruses can cause up to 30% crop reduction (Van Emden & Harrington, 2007; Ragsdale et al., 2001). Infected plant only shows symptoms in daughter tubers term net necrosis, leaf roll, systemic necrosis, leaf drop streak, dropping of lives and lost vigor and yield potential in the plant (Ragsdale et al., 2001; Novy et al., 2002).

GPA has developed resistance against most of the registered synthetic insecticides (Silva et al., 2013). A reduction of 80% of the crop yields was reported when no control measures were applied (Tolman et al.,2004). Alternatives to insecticides and sustainable control measures were required to avoid the rapid emergence of insect resistance. In addition to the increase of human and beneficial organism risk and the negative impact on the environmental (Speranza et al., 2008).

Biological control, transgenic crop, and integrated pest management (IPM) were alternatives to chemical control. However, the transgenic crop might not be allowed in some countries (Burketova et al., 2015). Industrialized countries were working on reducing the usage of pesticides worldwide by searching for alternative ways to control plant insect pests and diseases. The rapid increase of GPA population density tends to enhance the development of insecticide-resistant, which could be harmful to human health and the environment. Plant-induced resistance is new, safe, and innovative techniques to protect crops from insect infestation reduces crop losses, contribute to sustainable agriculture production and food security. (Speranza et al., 2008; Chandler et al., 2011). Inducing systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of inducing resistance dependent on protein. The most important for all plant's systemic responses treated plant by oil can lead to inducible defense proteins. Such researches are getting more attention and growing interest (Burketova et al., 2015; Yan et al., 2013; Conrath et al., 2002).

Essential oils are extracted from different plant parts (Moharramipour & Negahban, 2014), they are secondary metabolites that are highly volatile and have a strong odor (Bakkali et al., 2008). EOs could be used as alternatives to chemical pesticides; to reduce the negative impacts on the environment and could be safe for human and animal health (Liao et al., 2017; Kunbhar et al., 2018). EOs have a major role in the plant defense system against microorganisms, insects, and herbivores, they could induce SAR and ISR in the host plant (Heldt et al., 2005).

The Electronic Penetration Graph (EPG) has been developed to study piercing insect feeding, settling behaviors, and host selection by monitoring the stylist behavior within the plant tissues (Tjallinii & Esch, 1993). EPG principle is based on recording the voltage changes induced by feeding behaviors. Different feeding behaviors shows different waveforms pathways, through the mesophyll non-probing aphid stylist outside the plant (np), pathway activities (C), intercellular pathway punctures (Pd) associated with non- persistent virus inoculation, salivation associated with sieve element salivation or persistent virus inoculation) (E1), phloem-feeding and sieve element ingestion (E2), and G (xylem ingestion "drinking") (Halarewicz & Gabrys, 2012).

1.2 Objectives of the project

Therefore, the objectives of this project were to:

- Determine the response of potato crops to the indigenous medicinal Palestinian EO.
- Determine the response of GPA *M. persicae* to the indigenous Palestinian medicinal EO.
- Determine the effect of the indigenous Palestinian medicinal EO on potato plant induced defenses (POX and PPO).
- Monitor changes in aphid probing, feeding, and settling behavior to evaluate EO impact on aphid.

2 Literature review

2.1 Controlling Aphid pest *Myzus persicae*

Aphid is a major insect pest to many commercial crops during plant development, post-harvesting, and storage, thus they could reduce the yield of the crop (Dedryver et al., 2010).

Aphid control in potato has been primarily dependent on chemical control. It was reported that the extensive usage of chemical pesticides has led to enhance insect resistance and cross-resistance to pesticides (Stern et al., 1959). Also, the increase of public concern about their harmful impact on humans, animal health, and their high risk to the environment (Dedryver et al., 2010). Therefore, scientists are looking for an alternative method to chemical control with pesticides.

2.2 Essential oil usage

Previous studies have reported that many antimicrobial such as organic material, essential oil (secondary metabolite), microbial extracts, plant extraction, and seed oils are very important in medicinal research (Pillmoor et al., 1993).

Plant extractions and plant seed oils were used as insect pest repellent against harmful larvae of Lepidoptera, Coleoptera, Stored-Product Insects, and aphids (Ayvaz et al., 2010; Digilio et al., 2008). They could delay the immature development, adult emergence, and fertility (Marimuthu et al., 1997; Chakraborty & Chakraborti, 2010; Isman, 2000), this might be safe for human health and environment (Ahmed & Grainge, 1986; Tang et al., 2002).

Recent studies demonstrated the wide range of insect taxa that have been affected by different essential oils. Kanat & Alma,(2004) found insecticidal effects of essential oils from, *Lavandula stoechas* (L.), *Lavandula angustifolia* (L.), *Eucalyptus camaldulensis* (Dehnh.) and *Thymus vulgaris* (L.) against the larvae of pine processionary moth, Thaumetopoea pityocampa Schiff. The insecticidal effect of *Lavandula hybrid* (L.), *Rosmarinus officinalis* (L.), and *Eucalyptus globulus* (Labill.) oils were evaluated on *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) adults (Papachristos et al., 2004).

Tunc and Sahinkaya (1998) found that essential oils of cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisium* L.), oregano (*Origanum syriacum* var. bevanii L.) and eucalyptus (*E. camaldulensis*) were effective as fumigants against the cotton aphid (*Aphis gossypii* Glover).

2.3 Plant defense response

2.3.1 Plant induce resistant

Inducing resistance by stimulating the plant immune system is a recent innovation in crop protection which may replace insecticides used for pest control. Treating target plants with cell wall fragment, or plant extraction, or synthetic chemical that might be triggered upon infection of insect pests (Burketova et al., 2015; Lyon, 2007). The insect could also induce the production of different compounds such as polyphenol oxidase, peroxidase, lipoxygenase, and proteinase (Stout et al., 1997). Host plant resistance to insects, especially, induced resistance, could be manipulated with the use of chemical elicitors of the secondary metabolites, rapid release of Reactive Oxygen Species (ROS), production of toxins, antimicrobial compounds or enzymes, volatile organic compounds, which triggers resistance to insects (Chen, 2008).

War et al., (2015) found that the induction of enzyme activities of secondary metabolites was in all the insect-resistant genotypes infested with *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Phenolic compounds are the most common group of defensive compounds in secondary metabolites. They play an important role in plant resistance against insects (War et al., 2015; Rani & Jyothsna, 2010; Mazid et al., 2011; Kusnierczyk et al., 2008). They could accumulate by Plant growth-promoting Rhizobacteria (PGPR), or they could be directly toxic, or they could produce a hypersensitive response (HR) in plants (Sivasakthi et al., 2015; Kiprovski et al., 2016). Mobile signals are transmitted through the phloem from the induction site to plant tissues to protect it from insects. This is known as induce resistance such as systemic acquired resistance (SAR) or induce systemic resistance (ISR) (Bary, 2012)

2.3.2 Systemic acquired resistance (SAR)

SAR is a form of induced resistance in plants that mediates through the (SA) dependent pathway. SAR is an important signaling factor in the indication of plant-insect resistance and results in the accumulation level of defense hormone salicylic acid (SA) and activation of a wide range of genes called PR proteins (Fu & Dong, 2013; Vallad & Goodman, 2004). Treating plants with natural elicitors can induce resistance, and increase the potential to control insects associated with increased activities of plant defense-related enzyme contents, through inhibition of the digestive enzymes in the insect gut. Thus could replace pesticides (Fu & Dong, 2013).

2.3.3 Induced systemic resistance (ISR)

Induced systemic resistance (ISR) is the second main forms of induced resistance, by which selected plant growth-promoting bacteria and fungi in the rhizosphere prime of the whole plant body for enhanced defense against insect herbivores, and it depends on both the Jasmonic acid (JA) and Salicylic Acid (SA) signaling pathway figure (1) (Pieterse et al., 2014; Harun-Or-Rashid & Chung, 2017).

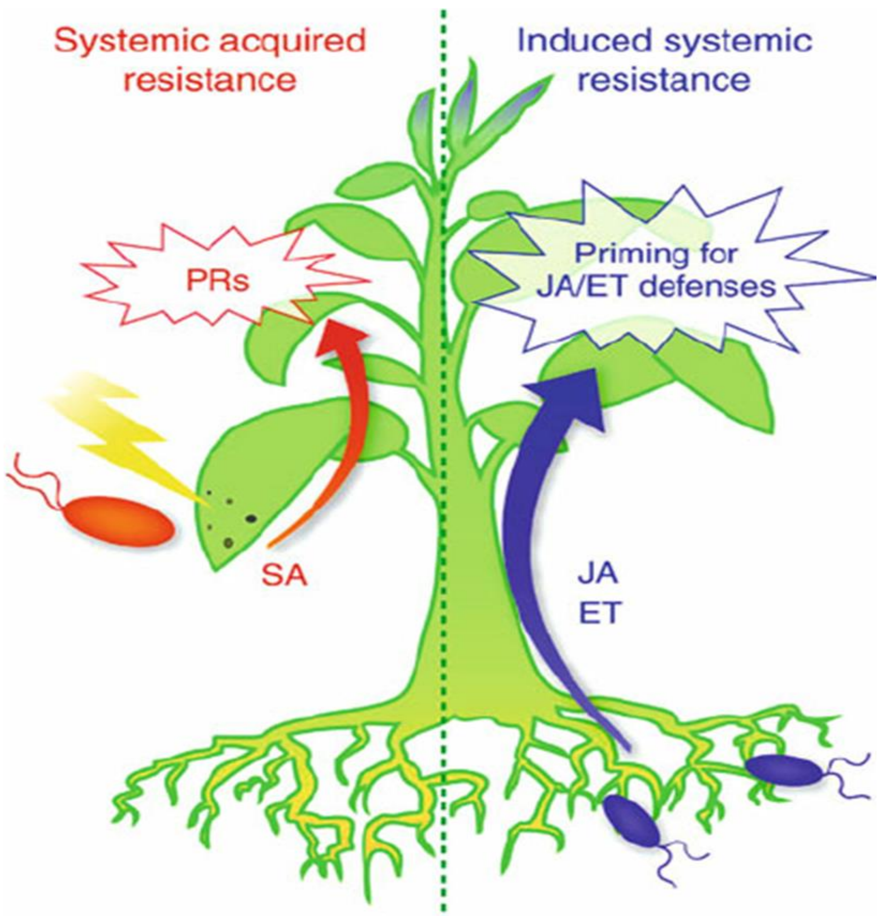

Figure 1: Systemic acquired resistance and Induced systemic resistance mechanism.

2.4 Electrical penetration graph (EPG)

The electrical penetration graph (EPG) is an important indicator and technique used to investigate the feeding behaviors of phloem- and xylem feeding insects such as whiteflies, scale insects, thrips, leafhoppers, and aphids. (Tjallingii & Esch, 1993). EPG allows real-time visualization of aphid feeding behavior. They are also used to determine plant resistance against sap-feeding insects. It was found that aphids taking a long time to locate the sieve element (SE), this could be associated with epidermis or mesophyll resistance; more cell punctures indicated problems in navigating through the plant tissues to the SE; shorter periods of ingestion from the SE (E2) showed a rejection of SE (Prado & Tjallingii, 1997).

EPG has been used to investigate the effects of pre-infestation by aphids on subsequent populations of the yellow sugarcane aphid, *Sipha Flava* (Forbes), results displayed improved feeding abilities on pre-infested *Sorghum halepense* (L.) leaves (Gonzales et al., 2002). AB Ghaffaret et al., (2011) used the EPG technique to study the feeding behavior of the planthopper *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae) on different rice varieties showing different levels of resistance.

3 Material and method

3.1 Insect colony and plant

GPA colony was initially brought to the laboratory from an infected potato plant collected from PTUK / Palestine. All stages of the aphid are maintained on the young potato plants for the laboratory trials following the methodology described by (Stobbs et al., 2015). The infected plants were propagated in a glasshouse at PTUK and maintained under standard conditions of 25 ± 5° C, 65 ± 5% relative humidity, and a photoperiod of 16h light, plants were fertilized with irrigation water weekly with Nitrogen: Phosphate: Potassium (NPK) (13:13:13). Soil combinations used in this study contained a mixture of 2:1of peat moss: vermiculite, then the mature aphids were kept on plants for 24 h, resulting in neonate nymphs with an age of 0–24 h that were used throughout the experiments.

3.2 Essential oil aphid Bioassay

Commercial preparations of 14 EO (Palsame essential oils, Jenin-Palestine) (Table 1) were used in this test. The insecticidal activities of the indigenous EO were evaluated against GPA. Newly laid aphids at day 0, neonate nymphs (aged 0–24 h) were obtained from the GPA population reared on potato plants. Nine aphids were transferred using a hairbrush to leaf discs cut by crock borer and dipped in EO concentration. Three leaf discs were placed in a 5 cm petri dish on a wet filter paper. Mortality was recorded after 72 h. For each EO, a stock solution was prepared with distilled water and diluted within a concentration range of (10,100, 500, 750, 1000 ppm). Five replicates were used for each EO tested, under room condition as shown in figure 2.

Table 1: List of the essential oils used in the study.

English Name	Scientific Name	Family
Thyme	*Thymus vulgaris*	Lamiaceae
Coriander	*Coriandrum sativum*	Apiaceae
Jojoba	*Simmondsia chinensis*	Simmondsiaceae
Nettle	*Urtica dioica*	Urticaceae
Sesame	*Sesamum indicum*	Pedaliaceae
Bitter Cucumber	*Colocynthis citrullus.*	Cucurbitaceae
Mustard	*Sinapis arvensis*	Brassicaceae
Eucalyptus	*Eucalyptus* globulus	Myrtaceae
Sage	*Salvia officinalis*	Lamiaceae
Rosmare	*Rosmarinus officinalis*	Lamiaceae
Camphor	*Cinnamomum camphora*	Lauraceae
Pomegranate	*Punica granatum*	*Lythraceae*
Mentha	*Mentha spicata*	Lamiaceae
Lavender	*Lavandula spica*	Lamiaceae

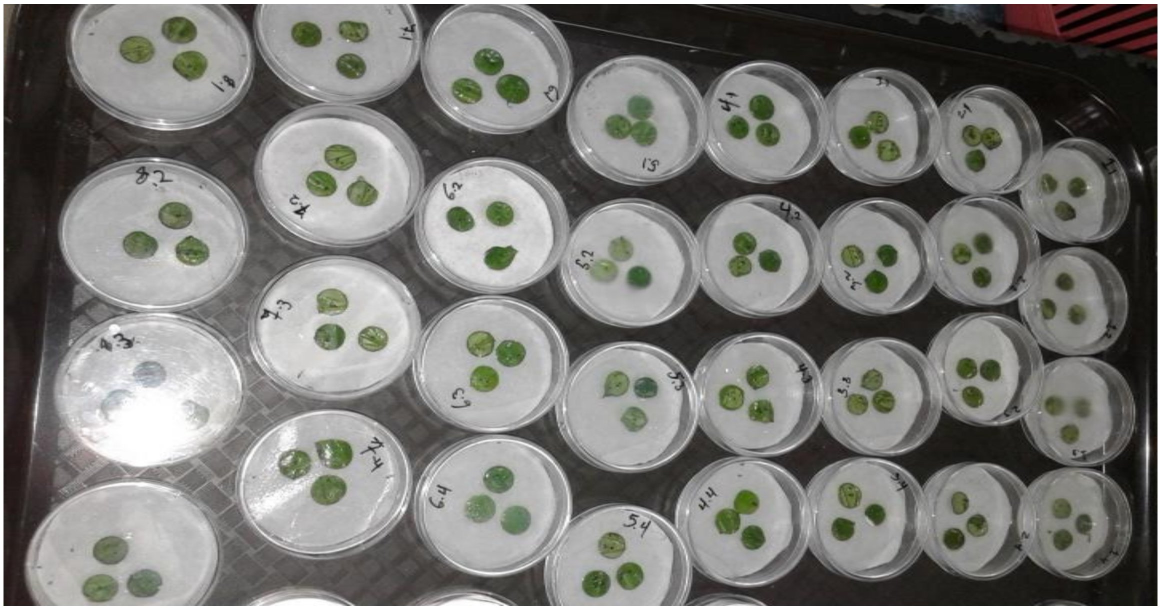

Figure 2: Essential oil aphid Bioassay within a concentration range of (10,100, 500, 750, 1000 ppm) after 72h.

3.3 Vegetative growth and yield

Potato plants were monitored to determine the effects of EO treatments. when the Potato plants were one week old (3-4 leaf stage plant), leaves were sprayed within (EO), and water for the control. During the growing period (five weeks) phytotoxic symptoms and shoot length were monitored weekly then plant were harvested to evaluate plant total yield per treatment, root growth, plant, and root fresh and dry matter where measured as described by Huang et al., (2019).

3.4 Enzyme Defense of Host Plant Assessment:

Assay of defense-related enzymes (POX and PPO) for the infected and control plant was measured. Peroxidase (POX) and polyphenol oxidase (PPO) activities were measured spectrophotometrically using (Hach Lange DR6000 UV-VIS Spectro-photometer, Germany). Potato “Sponeta” cultivar) with 5 replicate for each treatment at 1% concentrated after 48 h of treatment following the method of Scott et al., (2017). 0.3 g of frozen leaves sample (-80℃) were placed in 2 mL Eppendorf tube before homogenized with 1.25 µL of 0.1M potassium phosphate (K_3PO_4) buffer (pH 7, containing 7% (w:v) polyvinyl-pyrrolidone (PVP). Each homogenate extract was transferred to a new centrifuge tube. Then 100 µL of 10% solution of Triton X-100 was added with mixing vigorously around 10 seconds (sec). Tubes were centrifuged for 8000 rpm for 15 min using (Hettich® MIKRO 200/200R centrifuge, Z652121 SIGMA). Then, each supernatant was used immediately as an enzyme source for enzyme activity assay. All samples were kept on ice. To determine POX activity, 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 1 mL of freshly prepared 5 mM guaiacol with 0.02 mM hydrogen peroxide (H_2O_2) dissolved in 0.1M K_3PO_4 buffer pH 8 (Aman, 2015). For PPO assay 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 500 µL of fresh prepared 10 mM catechol dissolved in 0.1M K_3PO_4 buffer pH 8. Changes in absorbance were measured at 470 nanometers (nm) for 30 sec. at room temperature using a spectrophotometer device. Enzyme specific activity for both enzymes was reported as Absorbance/min/mg of fresh tissue weight (Boughton et al., 2006; Furumo & Furutani, 2008).

3.5 Electrical penetration graph (EPG)

Following the method of Tjallingii, (1988), the EPG system was used to monitor plant penetration and feeding activities of apterous adult GPA on potato seedlings sprayed with (1%) EO or water for the control. The equipment included an electrical resistor, a voltage source, and a Giga 8- DC EPG amplifier was set up (Martinet al., 1997). GPAs were starved for an hour then it was attached with fine gold wire with conductive silver paint and attached to the electrode; a second electrode, constructed of thicker copper wire, was placed in the soil of the experimental plant. The EPG was located within a faraday cage covered in silver foil to minimize interference and was run at a temperature of 24 ± 3°C. A voltage source and a 8- DC EPG amplifier with a 10^9 Ω input resistance. Eight channels were used concurrently for each adult GPA on potato seedlings sprayed with EO or water as the control. Each recording lasted for eight hours. All the signals were recorded and analyzed using STYLET+ software in figure 3.

Figure 3: Electrical Penetration Graph equipment

EPG waveforms have been previously characterized (Tjallingii & Esch, 1993). Typical traces displaying the main feeding behaviors are shown in Figure 4. the non-probing (NP; out of the plant); pathway (C; including cell penetrations); salivation in the SE (E1); SE sap removal (E2); xylem feeding (G) and difficulty feeding delay (F); number and the average length of potential drops (PDs), which represent cell penetrations.

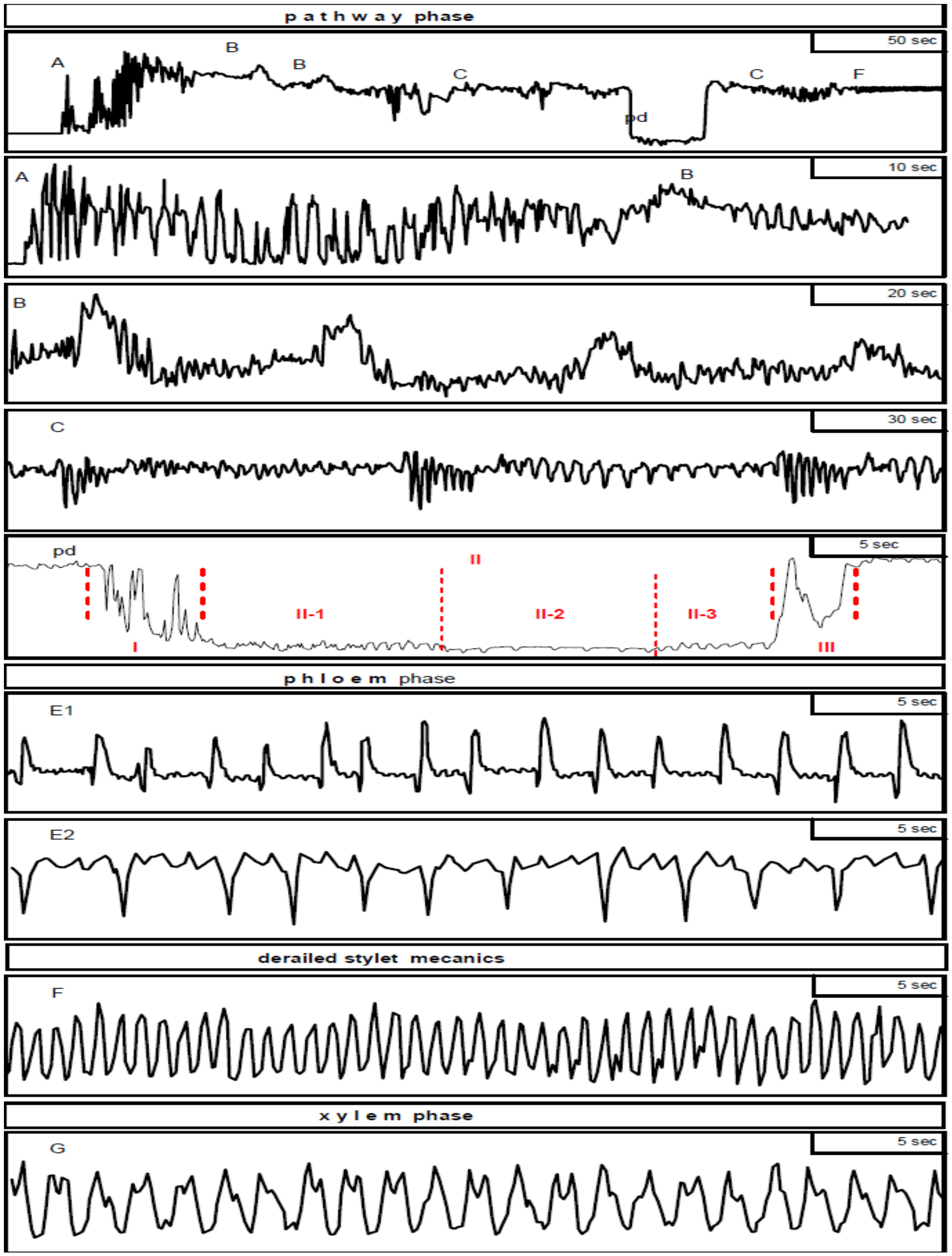

Figure 4: Summary of typical aphids waveform of electrical penetration graph. Non-probing (NP); out of the plant); Pathway (C); including cell penetrations); Salivation in the SE (E1); SE sap removal (E2); Xylem feeding (G) and difficulty feeding delay (F); number and the average length of Potential Drops (PDs), which represent cell penetrations.

3.6 Data collection and analysis method

Collection spread data sheets were analyzed using Analysis of variance (ANOVA) test using the general linear models procedure (PROC GLM). Levels of significance were determined by applying Duncan's multiple grouping test. On means and the standard deviation (Std) of 9 replicate readings at P= 0.001. Insecticidal activity bioassays of EO were calculated from probit regressions to measure LC_{50} and 95% confidence limits (Cl 95%). All statistical analyses were performed using Statistical Analysis System (SAS) (SAS Institute 2009). Figures and diagrams were carried out using (11, sigma plot system).

4 Results

4.1 Essential oil aphid Bioassay

Table 2 showed results of bioassay test using a different essential oil, the highest mortality rate (LC_{50}) was recorded for Sesame oil (1.06 ppm) followed by Mentha, Lavender, Rosemary, Pomegranate, Sage, Eucalyptus, and Bitter-Cucumber oil are (2, 3.3, 4, 6, 8.01, 8.01, and 10.04 ppm, respectively). Slope ranged Sesame, Mentha, Rosemary, Lavender, Pomegranate, Bitter-Cucumber, Sage, and, Eucalyptus are (0.01, 0.23, 0.29, 0.48, 0.5, 0.73, 6, and 6 ppm, respectively), The correlation of standard error had highly correlation and correlation of degree of freedom had highly correlated. Moreover, the toxicity for coriander was varied at LC_{50} 191 ppm with a slop 0.98, standard error 0.49, and degree of freedom 0.97.

4.2 Vegetative growth and yield

4.2.1 Vegetative and root growth of potato plant

Potato vegetative and root growth post EO treatment have no significant difference with control (Figure5A). Mustard oil recorded the highest growth of 50.5 cm compared with the control of 42.7 cm.

Table 2: LC_{50} values (with corresponding 95% confidence limits) for green peach aphid adults after 72 h exposure to potato leaf disks dipped in different concentrations of EO. Mortality in all control treatments was always below 10%. The results presented as LC_{50} with corresponding 95% confidence limits (CL), Pearson Chi-square results, degree of freedom (df), and regression equations.

OIL	Regression equations	X (df)	Slope ±Standard Error (SD)	LC_{50}(ppm)	(95% CL)
Thyme	0	0	0 ± 0	0	0
Coriander	y = 2.1659x + 1.5721	R^2 = 0.97 (4)	0.98 ±0.49	191	2.42489 - 5057
Jojoba	y = 8.2913x - 2.6852	R^2 = 0.97(4)	0.64 ±0.48	29	8.84325E-7 - 139.60434
Nettle	y = 5.31x - 1.3554	R^2 = 0.97(4)	0.89±0.49	20	0.55979 - 65.52865
Sesame	y = 6.0616x - 3.0045	R^2 = 0.97 (4)	0.01 ±0.54	1.06	3.2321E-11 - 11.33740
Bitter cucumber	y = 4.966x - 1.4811	R^2 = 0.97 (4)	0.73 ±0.50	10.04	0.15297 - 36.89183
Mustard	y = 7.0796x - 2.35	R^2 = 0.97(4)	0.61 ±0.48	15.47	0.00155 - 71.86417
Eucalyptus	y = 0.5599x + 0.6242	R^2 = 0.97 (4)	5.87 ±4.86	8.01	0
Sage	y = 0.5599x + 0.6242	R^2 = 0.97 (4)	5.87 ±4.86	8.01	0
Rosemary	y = 7.4217x - 3.1216	R^2 = 0.97 (4)	0.28 ±0.49	4	4.308E-10 - 30.32168
Camphora	y = 4.0745x - 0.7981	R^2 = 0.97(4)	1.10 ±0.50	17.344	1.71848 - 47.50174
Pomegranate	y = 5.6475x - 2.0474	R^2 = 0.97(4)	0.5 ±0.50	6	0.00512 - 29.13202
Mentha	y = 3.607x - 1.5774	R^2 = 0.97(4)	0.22±0.70	2	1.04174E-7 - 8.80587
Lavender	y = 3.9576x - 1.4602	R^2 = 0.97(4)	0.47±0.58	3.30	0.00804 - 13.94872

* Regression equations estimated by probit regression
** (95%) Confidence limits for LC_{50} in ppm

Camphor, Jojoba, Eucalyptus, Pomegranate, Sage, Rosemary, Lavender, Coriander, Bitter Cucumber, Mentha, and Nettle, have higher vegetative growth level by (50, 49.2, 49.1, 47.5, 46.8, 46.5, 46.5, 46.2, 45.6, 45.4 and 44.1 cm, respectively). Sesame oil had a decreasing range of length by 35.8 cm compared with the control. No data were recorded for plants treated with Thyme oil the plants showed wilting, chlorosis, and burning symptoms.

Rosemary treatment caused no significant increase in the root growth (24 cm) compared with the control (20.6 cm). Mustard, Lavender, Pomegranate, Mentha, and Sage, caused also a non-significant increase in root growth (22.6 to 22 cm) (Figure 5B). Eucalyptus, Nettle, Bitter Cucumber, Camphor, Coriander, Jojoba, and Sesame had a non-significant lower root growth to control (20.3, 19.6, 19.3, 19, 16.3, 15.6 and 15.3 cm, respectively). No data were recorded for plants treated with Thyme oil, as the plants showed wilting, chlorosis, and burning symptoms.

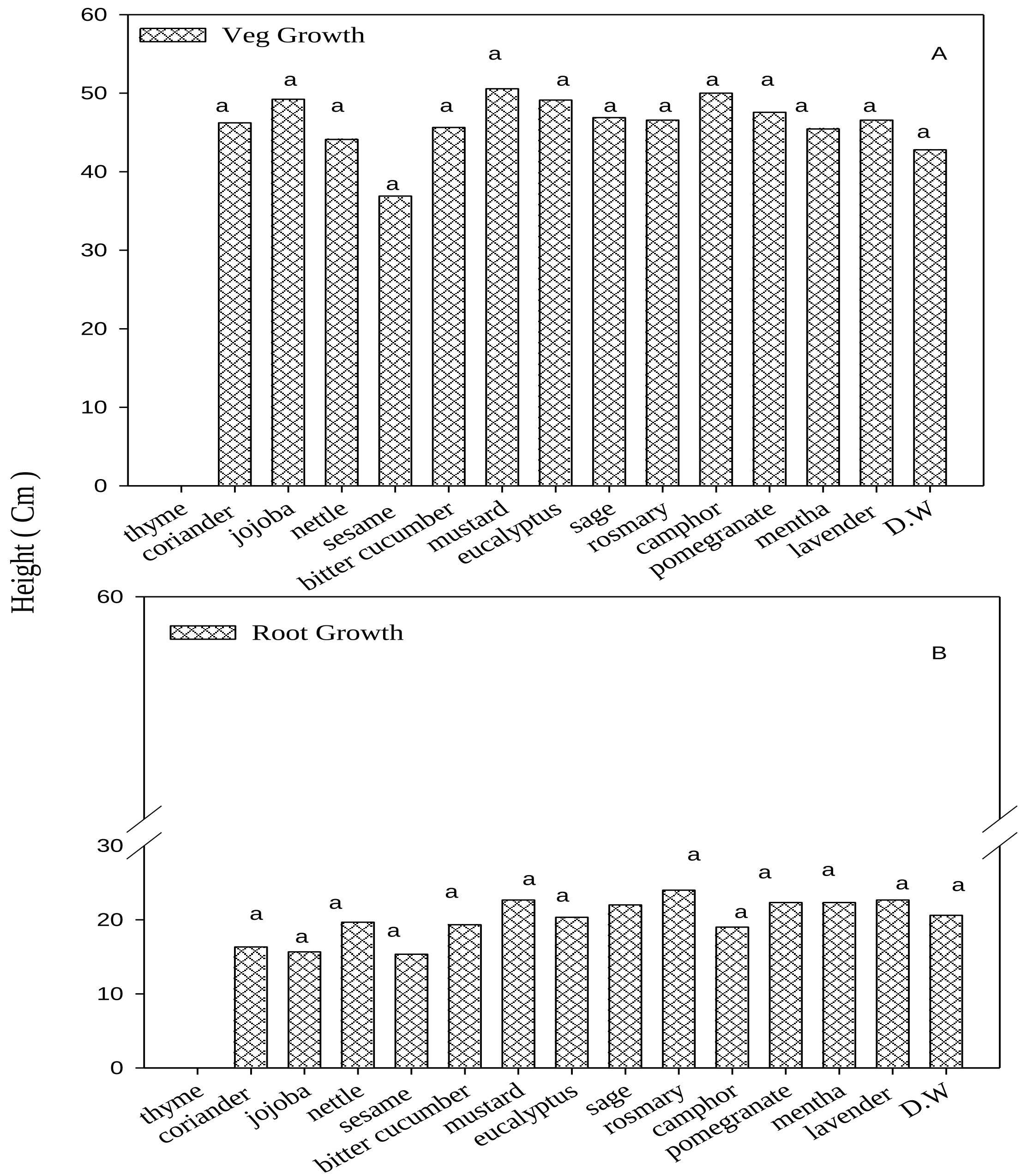

Figure 5: vegetative (A.) and root (B.) growth of potato plant after EO treatment, results represent mean and Std of 9 replicates. Similar letters represent the same impact at P= 0.001.

4.2.2 Tuber growth of potato plant

Results in Figure 6. showed that Mustard, Sesame, and Rosemary had a no-significant value on tuber number, approximately 10 tubers compared with the control (7 tubers). Followed by Coriander, Nettle, and Bitter Cucumber had a higher number of tubers (9 tubers), Sage and camphor had (8 tubers), and Eucalyptus, Pomegranate, and Mentha had a similar number as in the control. No data were collected for plants treated with Thyme oil as mentioned earlier.

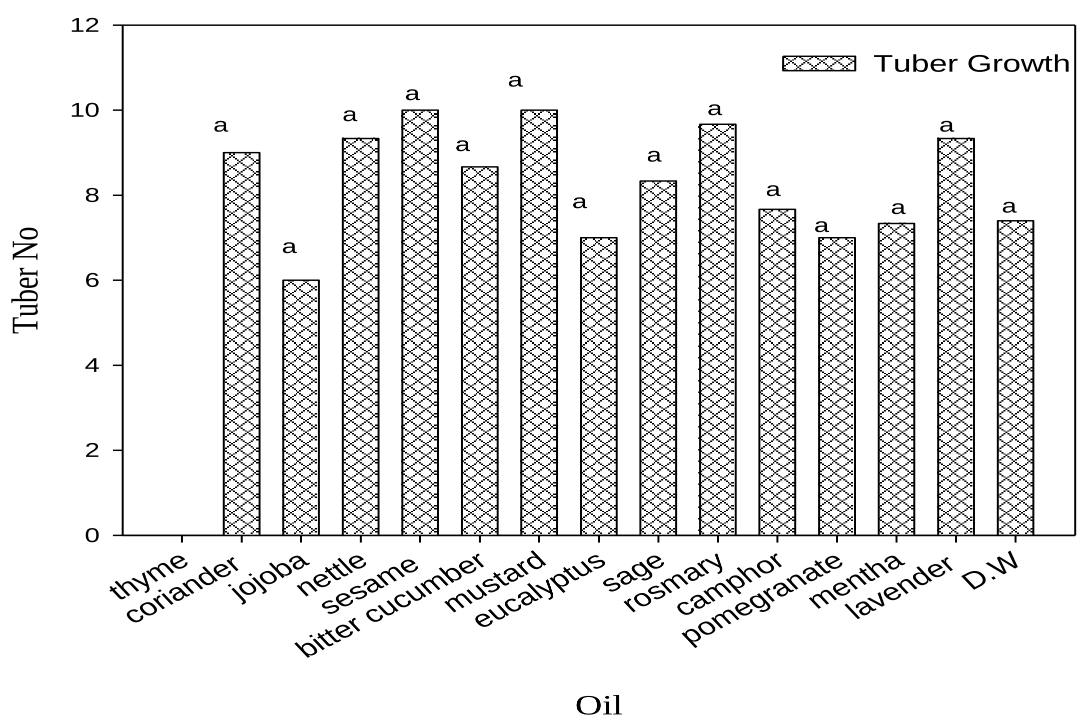

Figure 6: The number of tuber growth in potato plant after EO treatment, results represent mean and Std of 9 replicates. Similar letters represent the same impact at P= 0.001

4.2.3 Shoot fresh/ dry weight

Plants treated with EO showed a significantly higher shoot fresh and dry weight compared with control (Figure 7). Mustard, Pomegranate. Eculauptas, lavender, Mentha, Sage, Bitter Cucumber, Rosemary, Camphor, Jojoba, Coriander and Nettle, recorded significant increase effect on shoot fresh weight (23.21g 22.19, 22.08, 21.76, 20.34, 19.57, 17.59, 16.76, 16.48, 15.06, 14.52, and 13.95g, respectively (Figure 7A).

Results of EO impact on shoot dry weight showed significant differences of Mustard, Lavender, Eucalyptus, Pomegranate, Jojoba, Sage, Mentha, Rosemary, and Camphor (2.7,2.37, 3.63, 2.29, .2.22, 1.98, 1.97, 1.67, 1.59g respectively), compared to control (Figure 7B).

4.2.4 Root fresh/dry weight

Root fresh and dry weight of plants treated with EO showed no significant differences with the control (Figure 8A,8B).

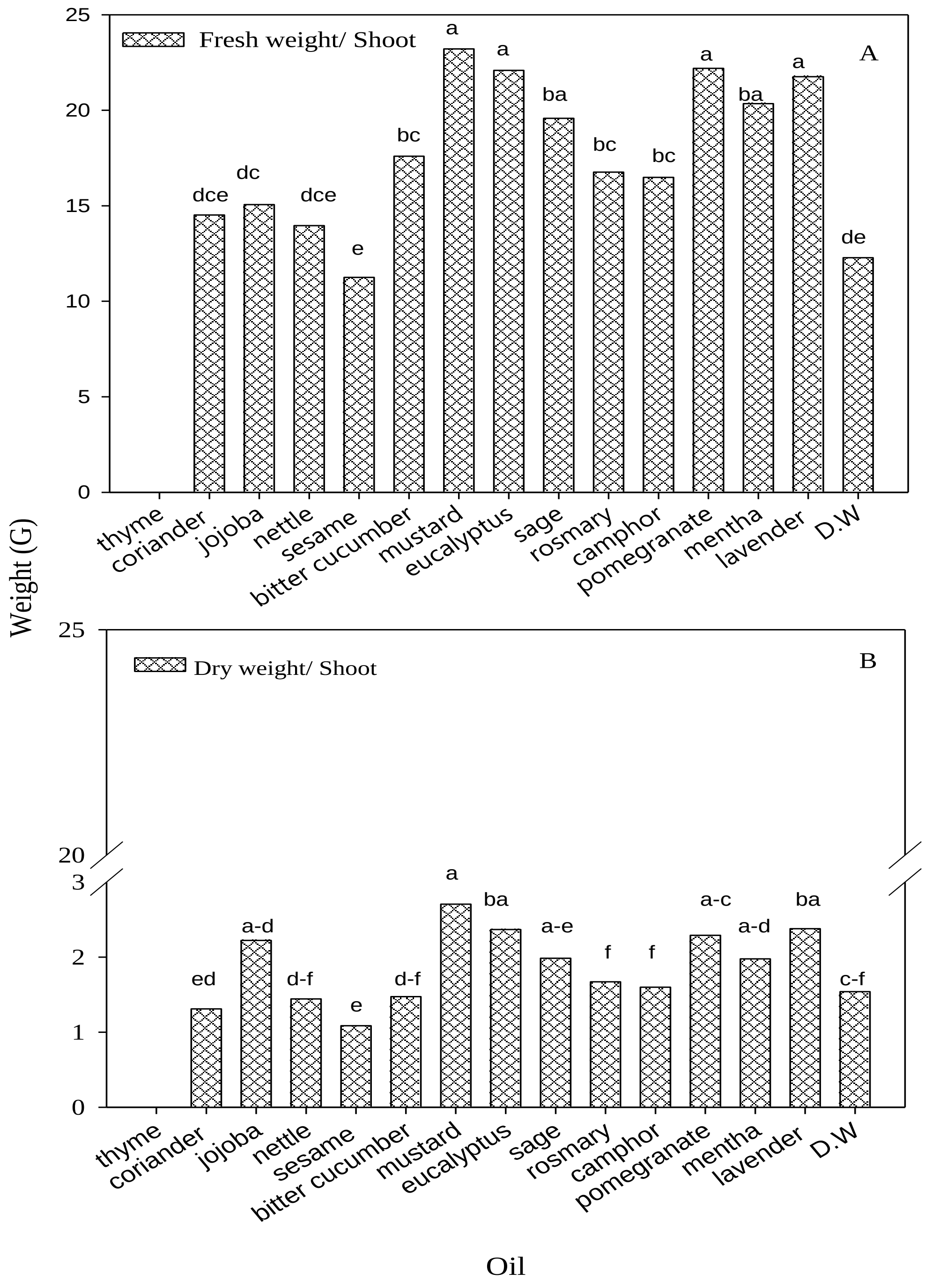

Figure 7: Shoot fresh (A.) and dry (B.) weight of potato plant after EO treatment, results represent mean and Std of 9 replicates. Similar letters represent the same impact at P= 0.001.

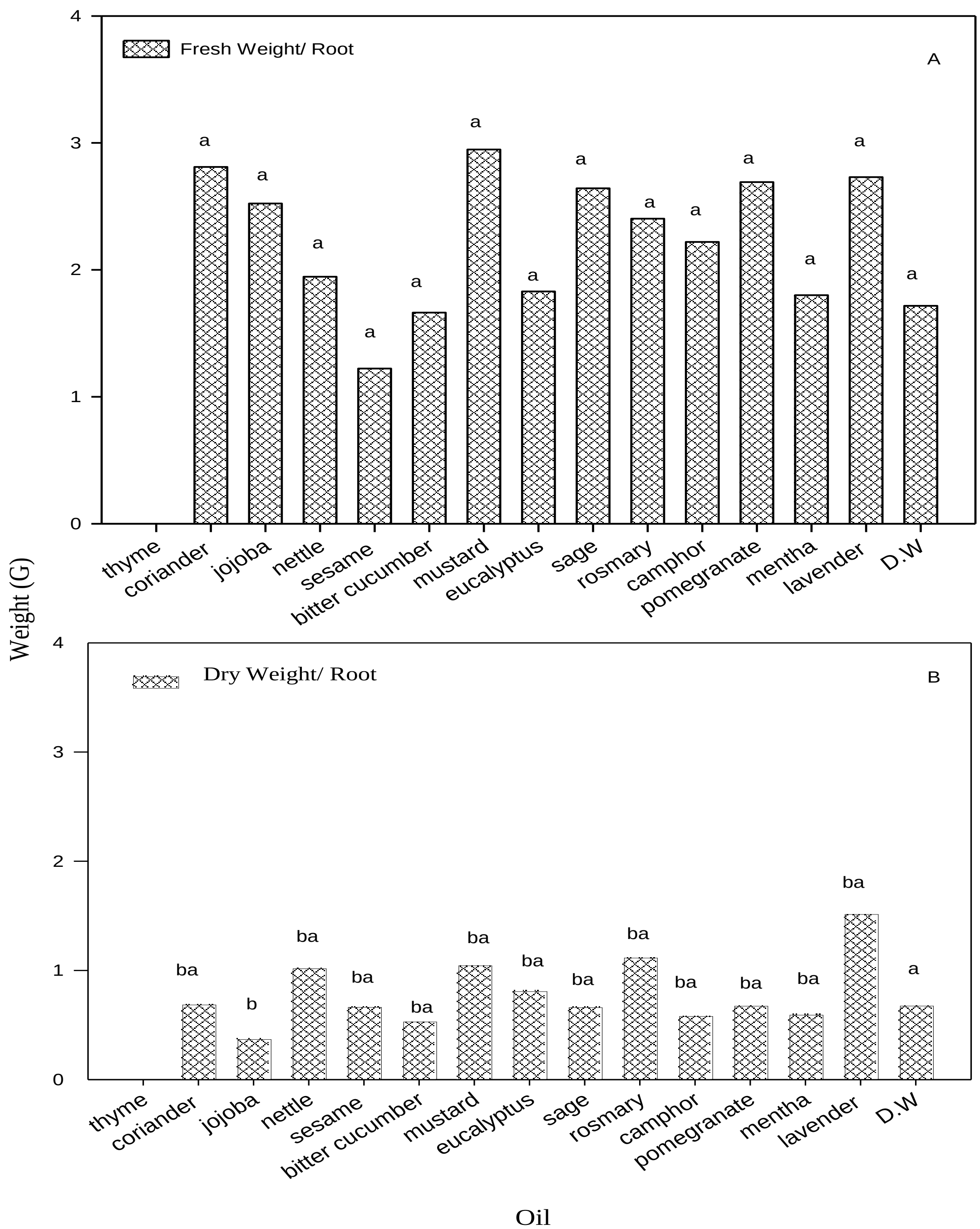

Figure 8: Root fresh (A.) and dry (B.) weight of potato plants after EO treatment, results represent mean and Std of 9 replicates. Similar letters represent the same impact at P= 0.001.

4.3 Assessment of EO treatment on enzyme activity of host plant

4.3.1 EO treatment impact on PPO of potato leaves

Plants treated with Jojoba showed high significant impact on PPO activity (0.98 OD/min/mg) compared with the control (0.58 OD/min/mg) (Figure 9). Followed by Coriander and Thyme, which had an increasing effect on the PPO level (0.71 and 0.66 OD/min/mg, respectively).On the other hand , Sesame, Nettle, Camphor, Lavender, Sage, Mentha, Rosemary, Pomegranate, Eucalyptus, and Mustard had a significant decreasing impact on PPO activity (0.54, 0.36, 0.26, 0.24, 0.22, 0.2, 0.16, 0.12, 0.1, and 0.08 OD/min/mg,respectively).

4.3.2 EO treatment impact on POX of potato leaves

Figure 10 showed POX readings for potato plants treated with EO. no significant impact of the different treatments on POX activity compared with control.

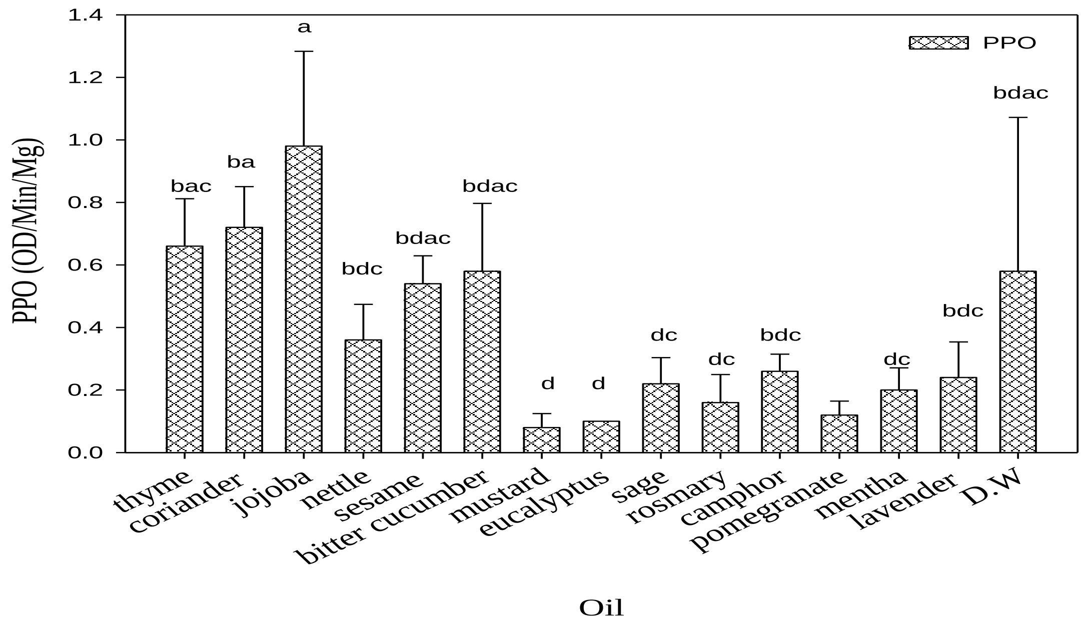

Figure 9: PPO activity in potato plant after EO treatment, results represent mean and Std of 5 replicates. Similar letters represent the same impact at P= 0.001.

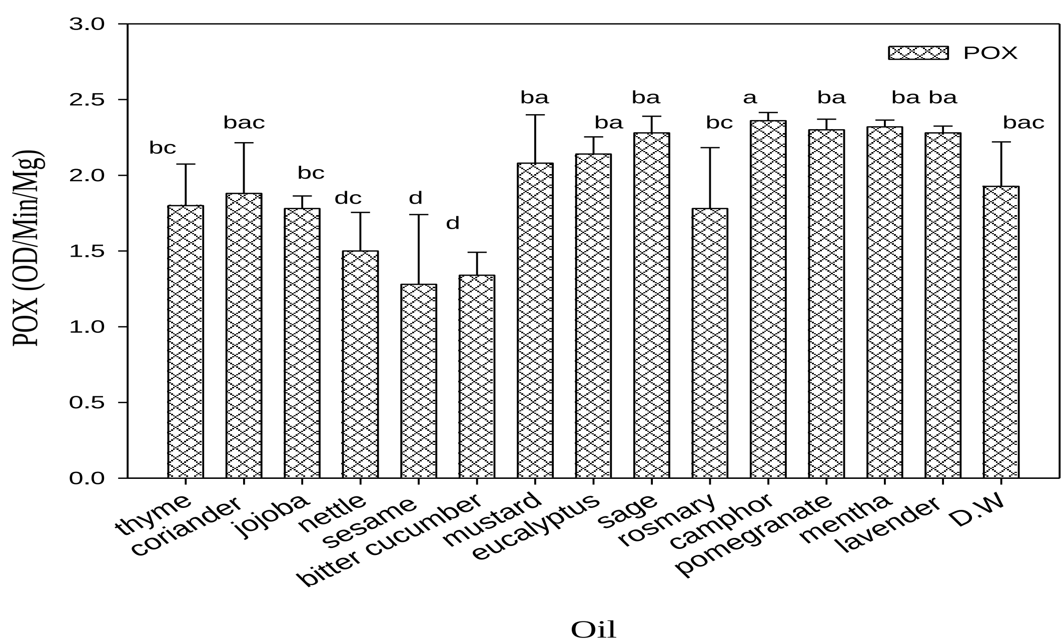

Figure 10: POX activity in potato plant after EO treatment, results represent mean and Std of 5 replicates. Similar letters represent the same impact at P= 0.001.

4.4 Electrical Penetration Graph (EPG)

The data for the electrical penetration graph followed a normal distribution (P > 0.001), so Duncan's multiple grouping test has been used for the analyses.

4.4.1 The first time of Aphids waveforms

Figure 11 illustrated that during the first two hour EPG recordings including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on plants treated with all EO compared with control. Although Pomegranate, Jojoba. Bitter Cucumber, Sage, Sesame, and Rosemary during the pathway behavior (729, 633, 579, 577, 536, and 520 sec., respectively) compared with control (429 sec) but was not significantly different.

Non-probing waveforms (NP), Camphor, Sage, Rosemary, Jojoba, Pomegranate, Coriander, and Mentha recorded the longest delay in GPA settling behavior (426, 393, 259, 203, 188, 152, and 151 sec., respectively) compared with the control.

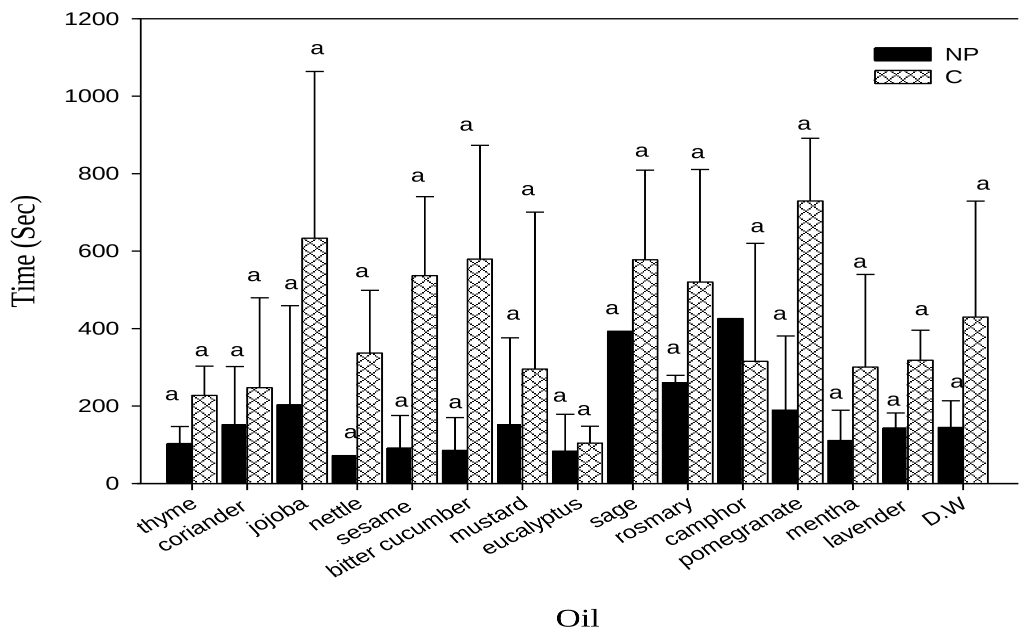

Figure 11: The Electrical Penetration Graph (EPG) was used to monitor the first time of feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Figure 12 Showed results of E1 (associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a non-significant delay between plants treated with EO and control. Sesame, Pomegranate, and Sage (5606, 3705, and 3075 sec., respectively Figure 12), While in SE sap removal (E2) EO treatments had no significant difference compared with control (Figure 12). Bitter cucumber, Mustard, Eucalyptus, Camphor, and Mentha prevented GPA sieve element behavior for both E1 and E2.

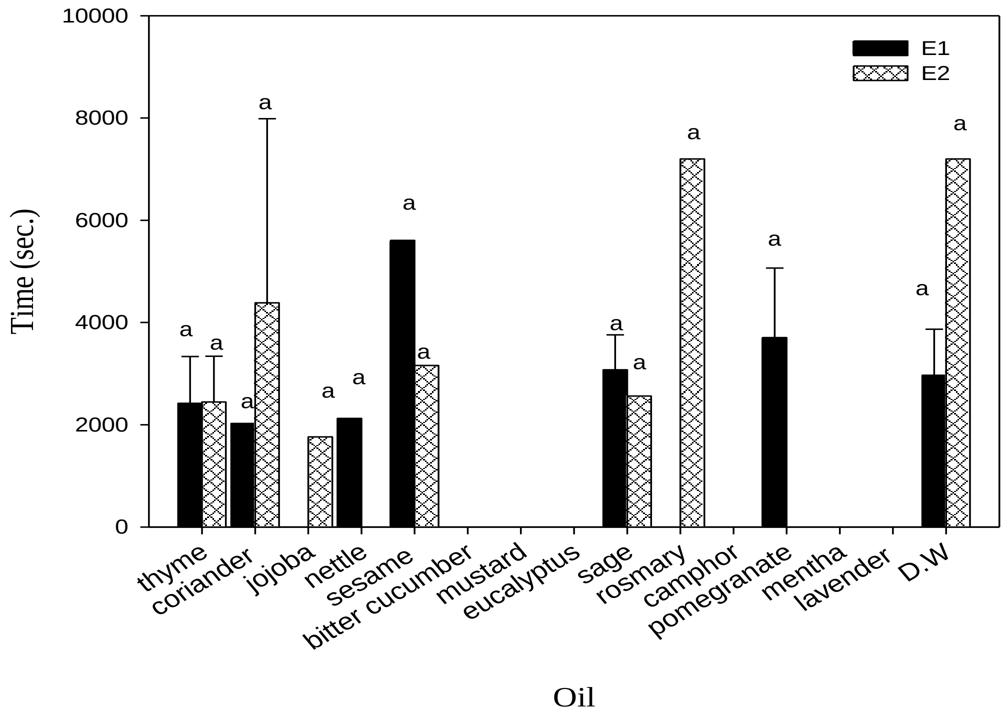

Figure 12: The Electrical Penetration Graph (EPG) was used to monitor the first time of feeding behaviors E1and E1 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

During the first two hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 13). In (G) waveform a non-significant impact of Jojoba, Rosemary, Thyme, Mentha, Sage (2736, 2671, 2598, 2096, and 2095 sec., respectively) compared with control (1699 sec.) (Figure 13). However, Bitter-Cucumber, Mustard, Eucalyptus, Camphor, and Lavender prevented GPA xylem feeding behavior (Figure 13). EO treatments showed a non-significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Mustard, Sage, Nettle, Eucalyptus, Thyme, Pomegranate, Coriander, Mentha, Rosemary, Sesame, Bitter Cucumber, and Jojoba (2724, 2603, 2463, 2410, 2239, 2021, 1408, 1279, 1249, 996, 959, and 944 sec,.respectively).

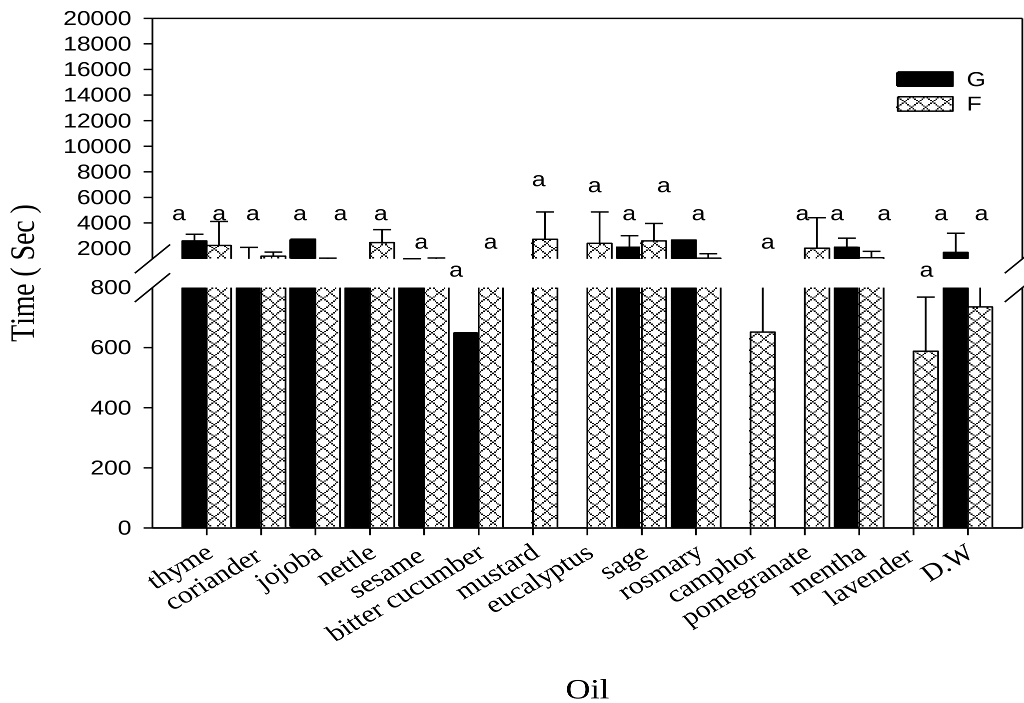

Figure 13: The Electrical Penetration Graph (EPG) was used to monitor the first time of feeding behaviors G and F waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

The potential drop of GPA behavior of the three sub-phases related to intracellular punctures associated with the acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 14).

The electrical penetration graph record during the first two hours showed that EOs treatments have a significant impact on PD (Figure 14). Mustard oil recorded the highest reading (2830 sec.) compared with the control (537 sec.) followed by lavender, Pomogranat, Nettle, Jojoba, Sesame, and Sage (1315, 1236, 925, 658, 611, and 600 sec respectively).

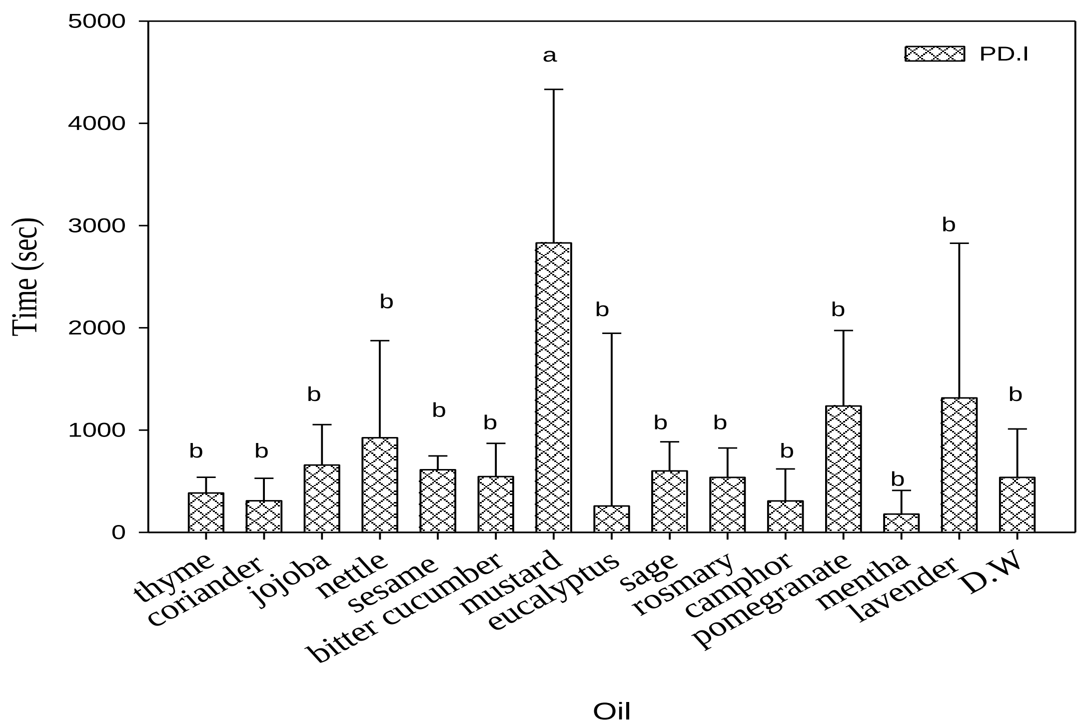

Figure 14: The Electrical Penetration Graph (EPG) was used to monitor the first time of feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

4.4.2The second time of Aphids waveforms

Figure 15 illustrated that during the first two hour EPG recordings including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on planter treated with all EO compared with their control. Although Sage, Bitter cucumber, Sesame, Rosemary, Mustard, Jojoba, Pomegranate, Mentha, Nettle, Lavender, Thyme, Camphor prolong the pathway behavior (690, 660, 655, 648, 633, 609, 552, 508, 507, 454, 435, and 340 sec., respectively) compared with control (327 sec), but was not significantly different.

Non-probing waveforms (NP), Bitter-Cucumber Mentha, Pomegranate, and Lavender recorded the longest delay in GPA settling behavior (3000,2490, 794, and 491 sec., respectively) compared with the control (383 sec). Oil Jojoba had the highest decreasing significant reading of 8 sec compared with water control, Moreover, the oil Thyme, Coriander, Nettle, Sesame, Mustard, Eucalyptus, Sage, Rosemary, and Camphor did not show any result, when they are compared with water control.

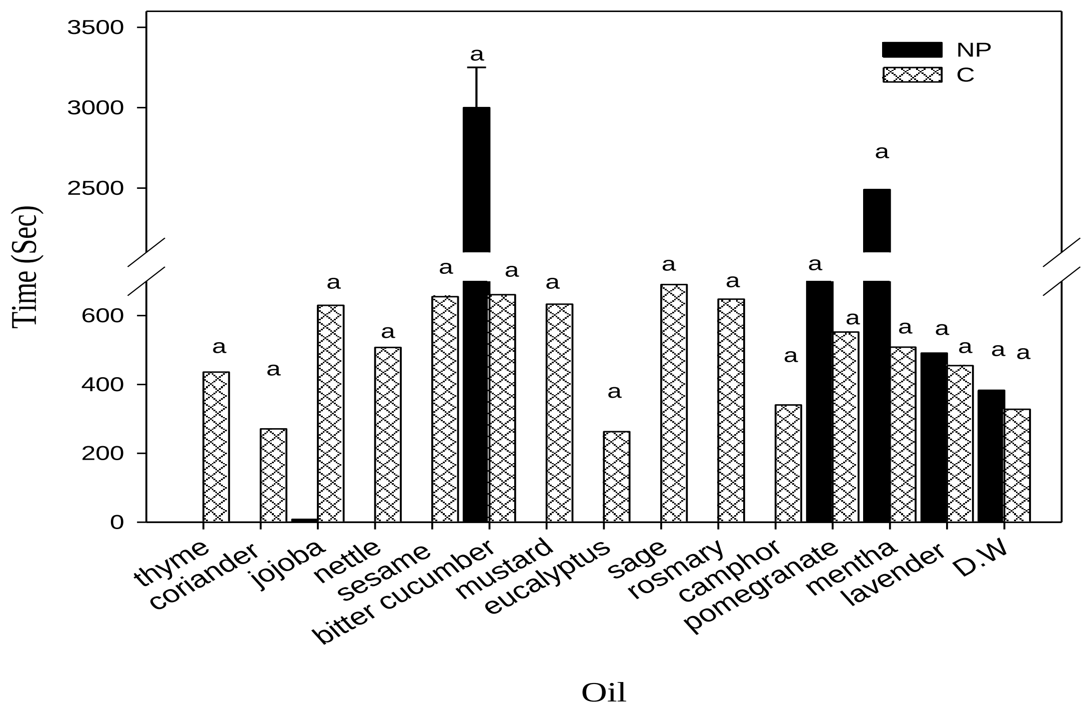

Figure 15: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Figure 16 showed the results of E1(associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a significant delay between plants treated with EO and control. Sesame, and Sage with readings of (5859 and 5539 sec) compared with control (4027 sec). While in E1 sap removal (E2) EO treatments had no difference compared with control (Figure 16). Bitter cucumber, Mustard, Eucalyptus, Rosemary, Camphor, Pomegranate, Menthe, and Lavender, prevented GPA sieve element behavior for both E1 and E2.

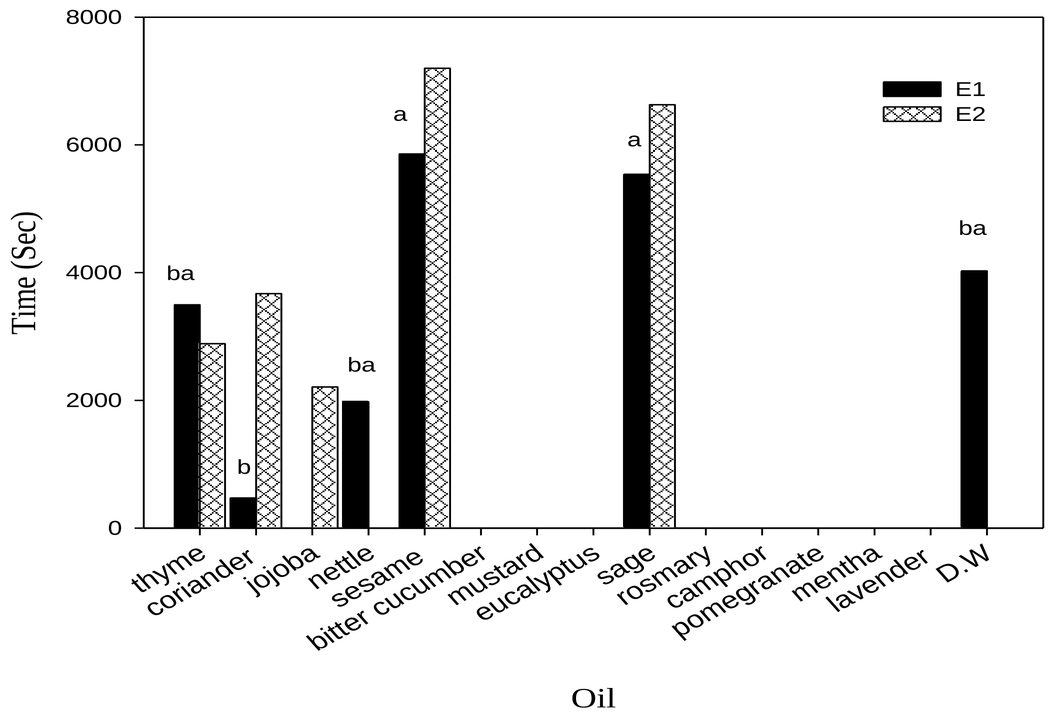

Figure 16: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors E1 and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P=0.001, The absence of identical characters due to the absence of duplicates.

During the first two hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 17). In (G) waveform a non-significant increment impact of Bitter Cucumber, Nettle, Mentha, and Sage (5290, 3100, 1995, and 1740 Sec., respectively) compared with control (1534 sec.) (Figure17). However, Thyme, Jojoba, Mustard, Eucalyptus, Rosemary, Pomegranate, and Lavender prevented GPA xylem feeding behavior (Figure 17). EO treatments showed a non-significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Mustard, Coriander, Sage, Rosemary, Thyme, Nettle, Pomegranate, Eucalyptus, Jojoba, Sesame, Mentha, and, Bitter Cucumber (2997, 3140, 2568, 2539, 2438, 2105, 1953, 1866, 1578, 1529,1494, and 1474sec., respectively).

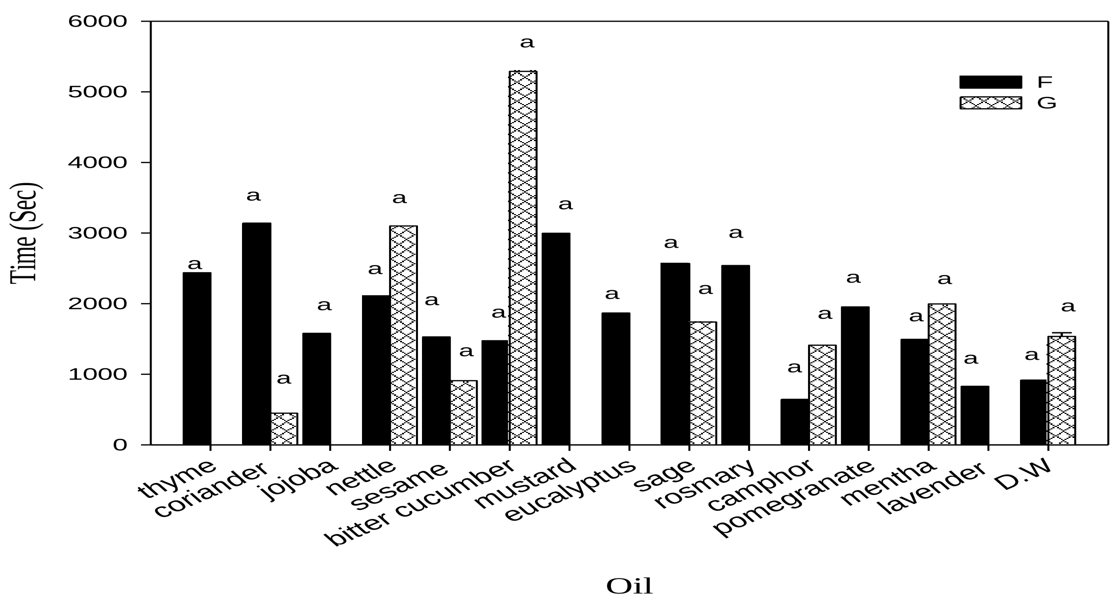

Figure 17: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors G and F waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 18).

The electrical penetration graph record during the first two hours showed that EOs treatments have a significant impact on PD (Figure 18), Mustard and Eucalyptus oils recorded the highest reading of (2917 sec.) compared with the control (589) sec.) followed by Pomegranate, Sage, Lavender, Bitter cucumber, Sesame, and Jojoba (2002, 1471, 1323,1209, 869, and 821 sec,respectively).

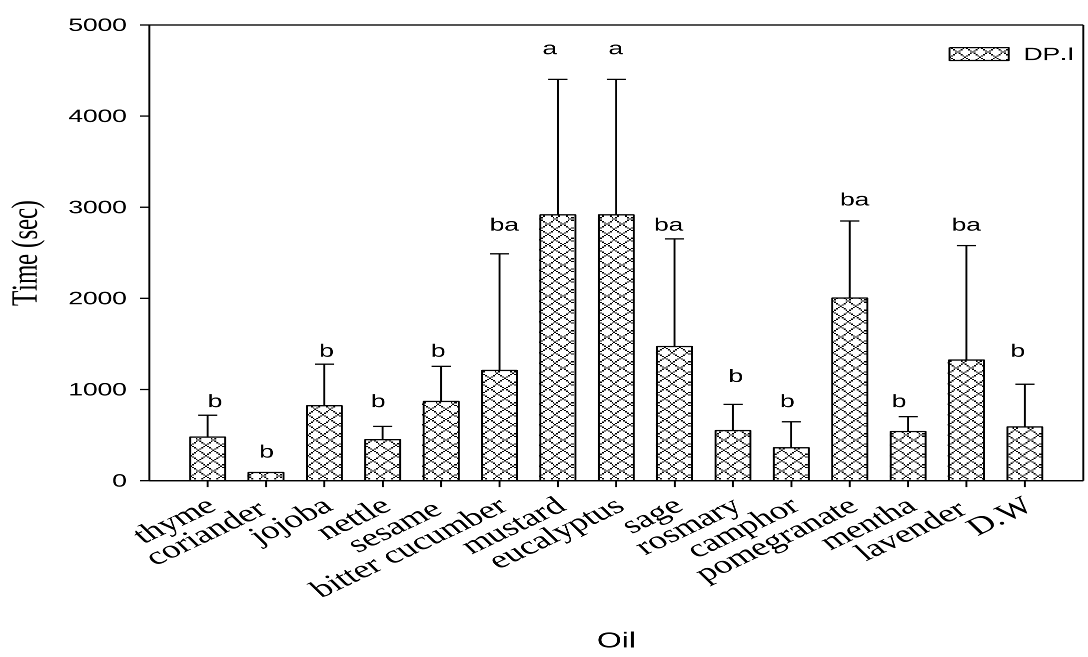

Figure 18: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P=0.001.

4.4.3 The third time of Aphids waveforms

Figure 19 illustrated that during the first two hour EPG recordings including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on plants treated with all EO compared with control. Although Eucalyptus, Sage, Jojoba, Pomegranate, Sesame, Mustard, Bitter Cucumber, Rosemary, Mentha, Lavender, Nettle, and Thyme prolong pathway behavior (887, 780, 698, 683, 678, 659, 605, 592, 574, 550, 549, and 512 sec., respectively) compared with control (503 sec), While Coriander and Camphor's readings had no significant effect compared to controls.

Non-probing waveforms (NP), Bitter Cucumber, Lavender, and Pomegranate recorded the longest delay in settling behavior (5558, 1409, and 1278 sec., respectively) compared with control (540 sec). Moreover, the oil Thyme, Coriander, Jojoba, Nettle, Sesame, Mustard, Eucalyptus, Sage, Rosemary, Camphor, Mentha, and Lavender did not show any result, compared with water control.

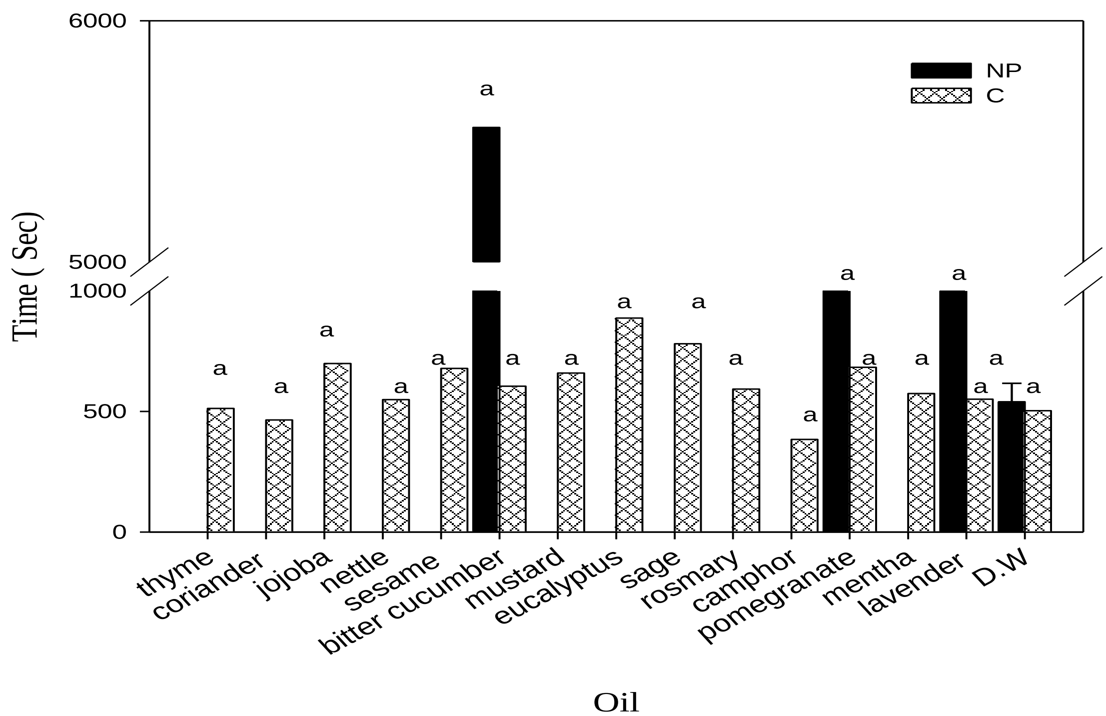

Figure 19: The Electrical Penetration Graph (EPG) was used to monitor second time feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001

Figure 20 showed the results of E1 (associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a non-significant delay between plants treated with EO and control. Coriander and Sage with readings (7187and 6021 Sec) compared with control (4679 sec). while in SE sap removal (E2) oil Jojoba which had an increasing effect on time level reading 2258 sec comparing with control and other oils did not show any result.

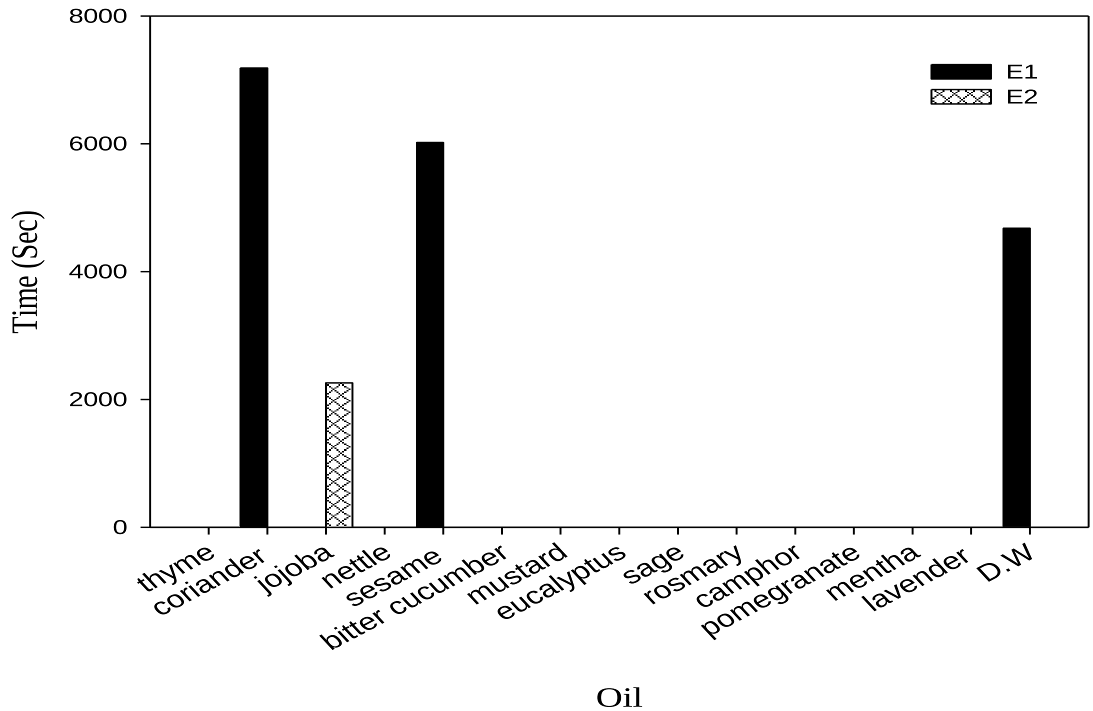

Figure 20: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors E1 and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001. The absence of identical characters due to the absence of duplicates.

During the first two hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 21). In (G) waveform no-significant impact of Mentha (3384sec) compared with control (2547sec). while oil Sage and Camphor had to decrease significant reading (2211 and 1653 sec) Moreover, all other oil did not show any result, when they are compared with water control.

While in (F) oil Mustard had a maximum reading 3839sec compared with control 2006 sec followed by, Bitter Cucumber, Rosemary, Sage, Pomegranate, Thyme, Coriander, Jojoba, and Eucalyptus(3232, 3150, 3139, 3105, 2943, 2745, 2482, 2205, and 2124 sec respectively). Lavender and Camphor and Mentha reading 1041, 961, and 679sec, respectively, were lower than the control.

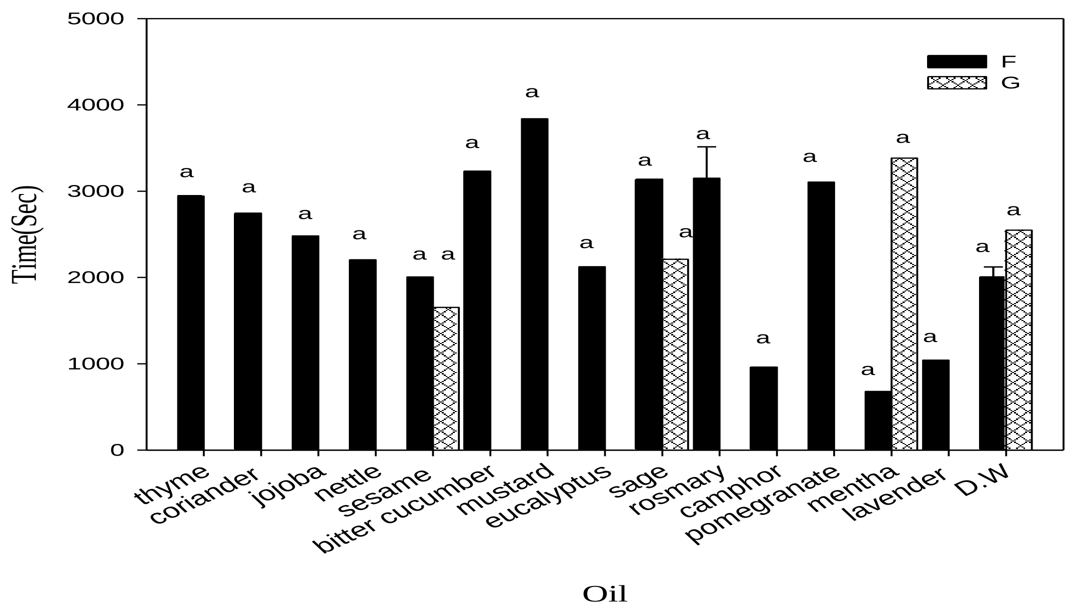

Figure 21: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors G and F waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 22).

The electrical penetration graph record during the first two hours showed that EO treatments have a significant impact on PD (Figure 22) Mustard oil showed the highest reading of (3151 sec.) compared with the control reading of (686 sec.) followed by Pomogranat, Sage, lavender, Eucalyptus, Bitter cucumber, Sesame, and Jojoba (2852, 1564, 1437, 1359, 1274, 1099, 1051, and 853 sec, respectively).

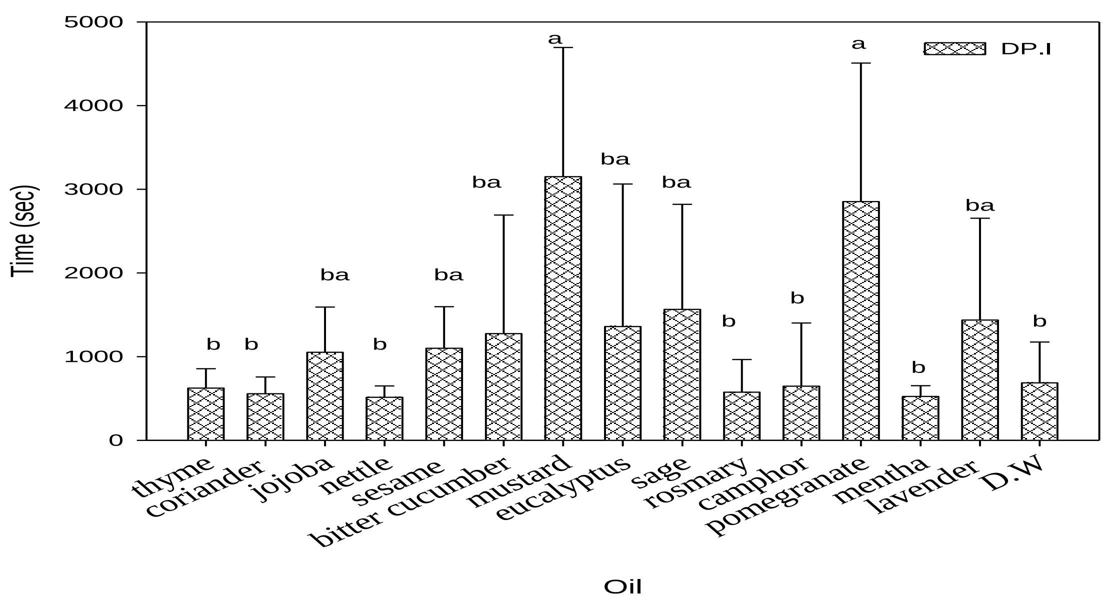

Figure 22: The Electrical Penetration Graph (EPG) was used to monitor the second time of feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001

4.4.4The duration of Aphids waveforms on first two-hour recording

Figure 23 illustrated that during the first two hour EPG recordings` including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on plants treated with all EO compared with control. Although Thyme, Coriander, Sesame, nettle, Rosemary, Sage, and Jojoba prolong the pathway behavior (2267, 2260, 1905, 1429, 1419, 1384, and 1061 sec., respectively) compared with control of (1037 sec).

Non-probing waveforms (NP), of plants treated with EO, showed no significant differences with the control.

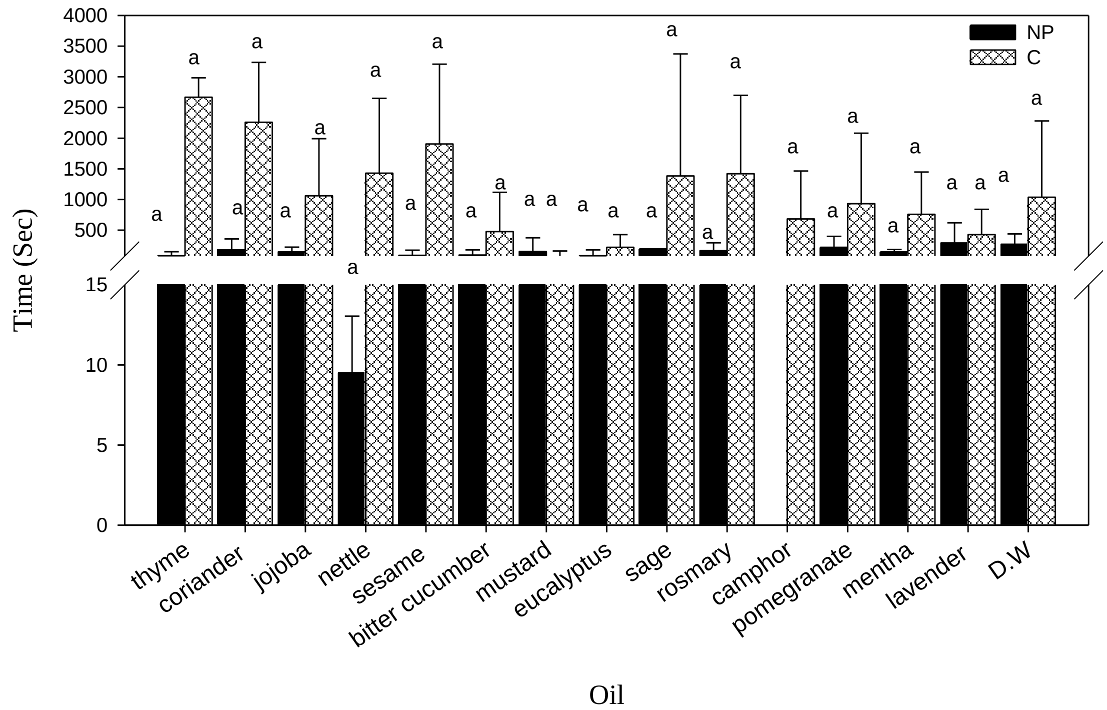

Figure 23: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Figure 24 showed results of E1 (associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a non-significant difference between plants treated with EO and control. Bitter cucumber, Mustard, Eucalyptus, Camphor, Mentha, and Lavender, prevented GPA sieve element behavior for both E1 and E2

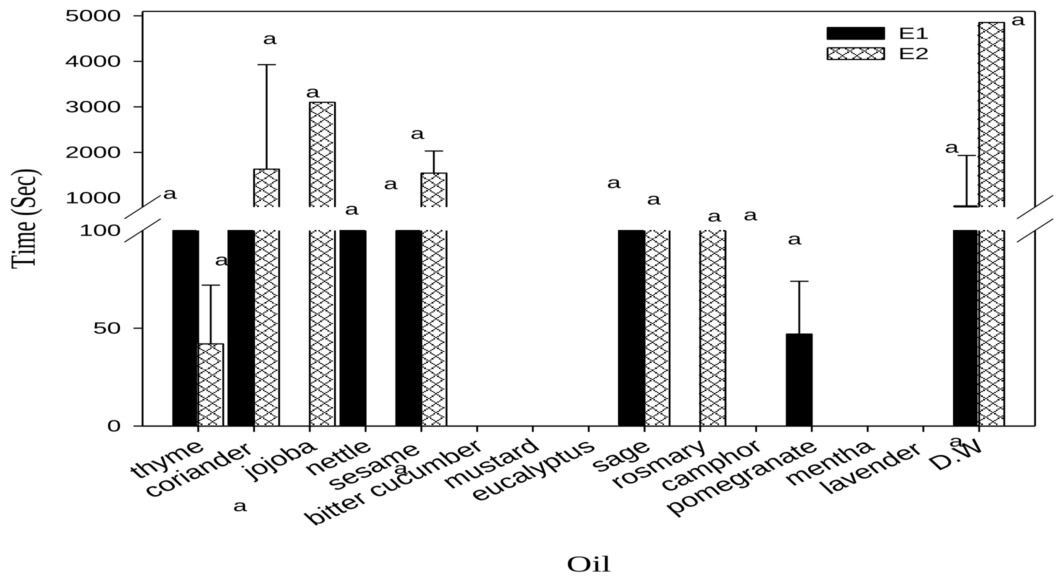

Figure 24: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors E1 and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001

During the first two hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 25). In (G) waveform a non-significant impact of Sage reading (48sec) compared with control (23sec.) (Figure25.A).

EO treatments (Figure 25.B) showed a significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Eucalyptus, Mentha, Pomegranate, Jojoba, and, Sage (7019, 3232, 3150, 3139, 3105, 2943, 2745, 2482, 2205, and 2124, sec., respectively).

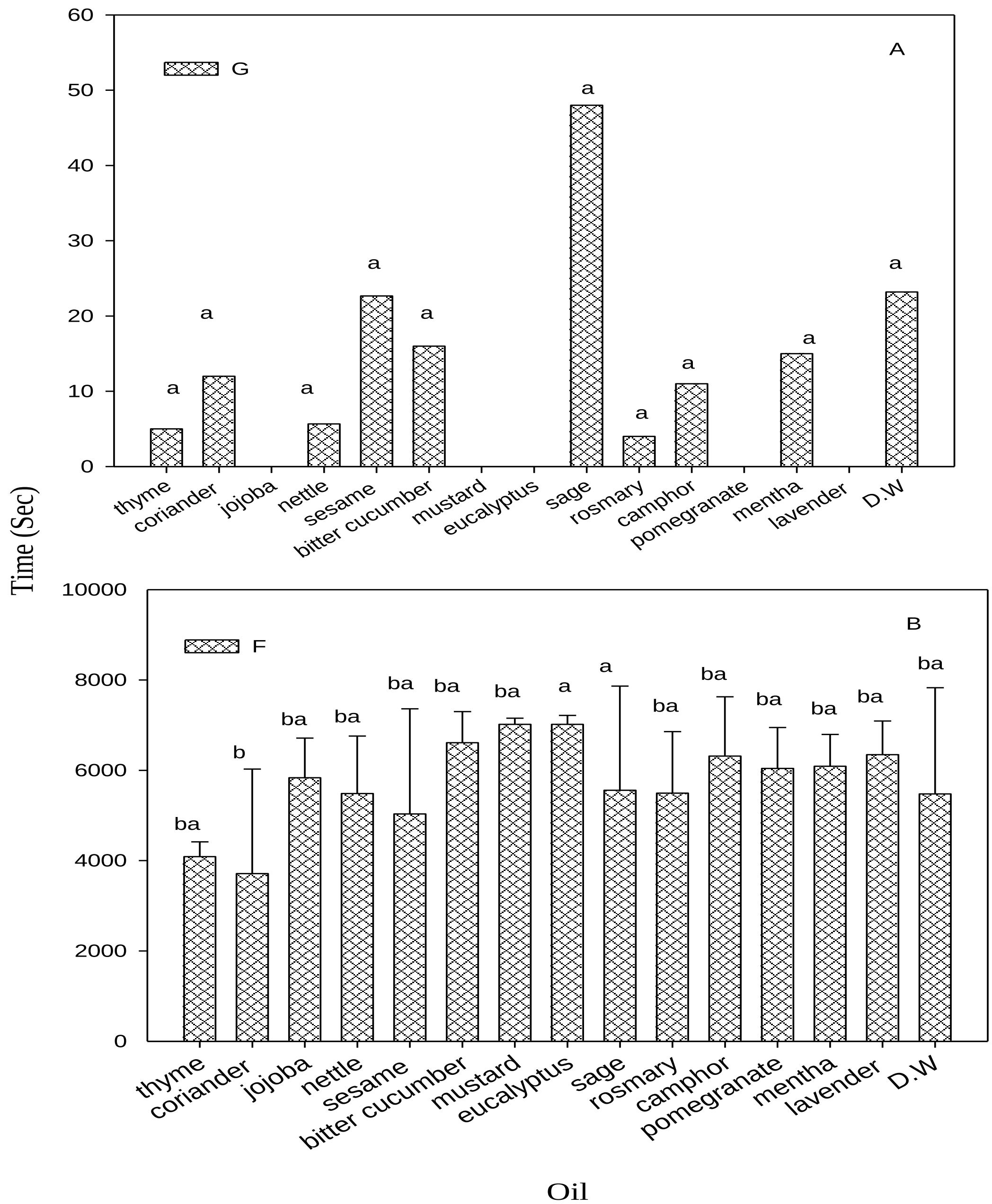

Figure 25: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors G(A) and F(b) waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 26).

The electrical penetration graph record during the first two hours showed that EO treatments have a significant impact on PD (Figure 26), lavender oil recorded the highest reading of (139 sec) compared with the control (37sec.) followed by Mentha, Rosemary, Camphor, Bitter Cucumber, Coriander, Mustard, Sesame, Jojoba, Pomegranate, Nettle, and Sage (162, 97, 88, 80, 75, 71, 68,67, 50, 49, and 47sec., respectively).

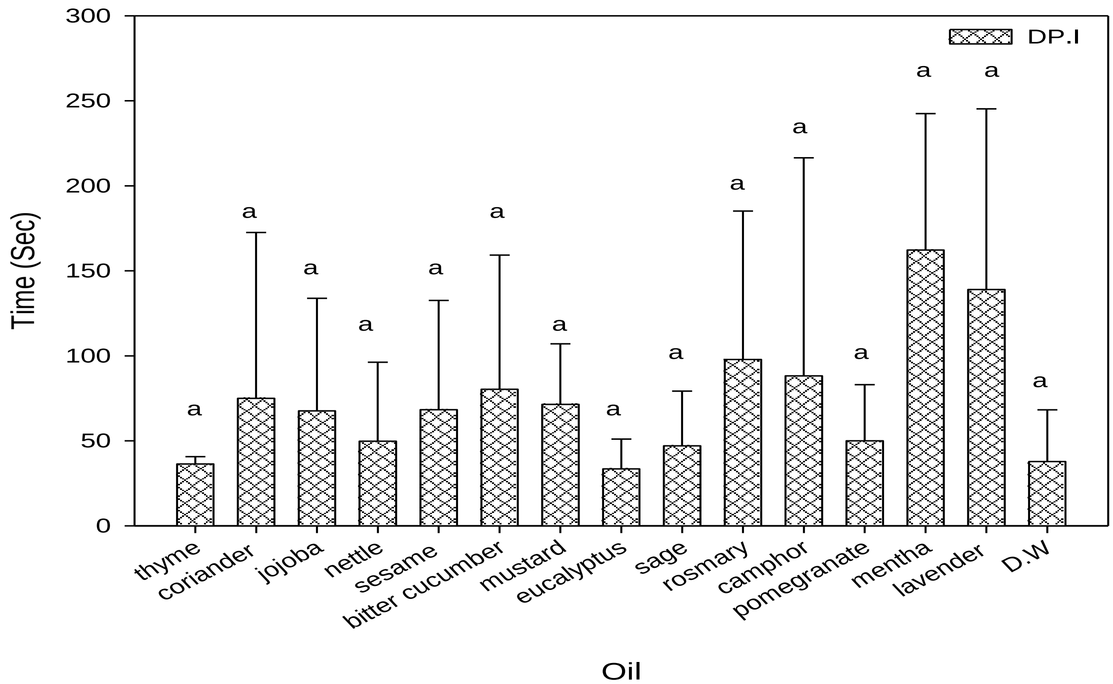

Figure 26: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

4.4.5 The frequency of Aphids waveforms on first two-hour recording

Figure 27 illustrated that during the first two hour EPG recordings includes pathway and non-probing behavior. In pathway waveform (C) reported a significant impact on plants treated with all EO compared with control. Although Thyme, Coriander, Eucalyptus, Sesame, Jojoba, Nettle, Rosemary, Lavender, and pomegranate (68, 63, 39, 33, 31, 29, 27, 25, 18, and 17 sec., respectively) compared with control (15 sec). While in non-probing waveforms (NP) no significant difference between all EO and control.

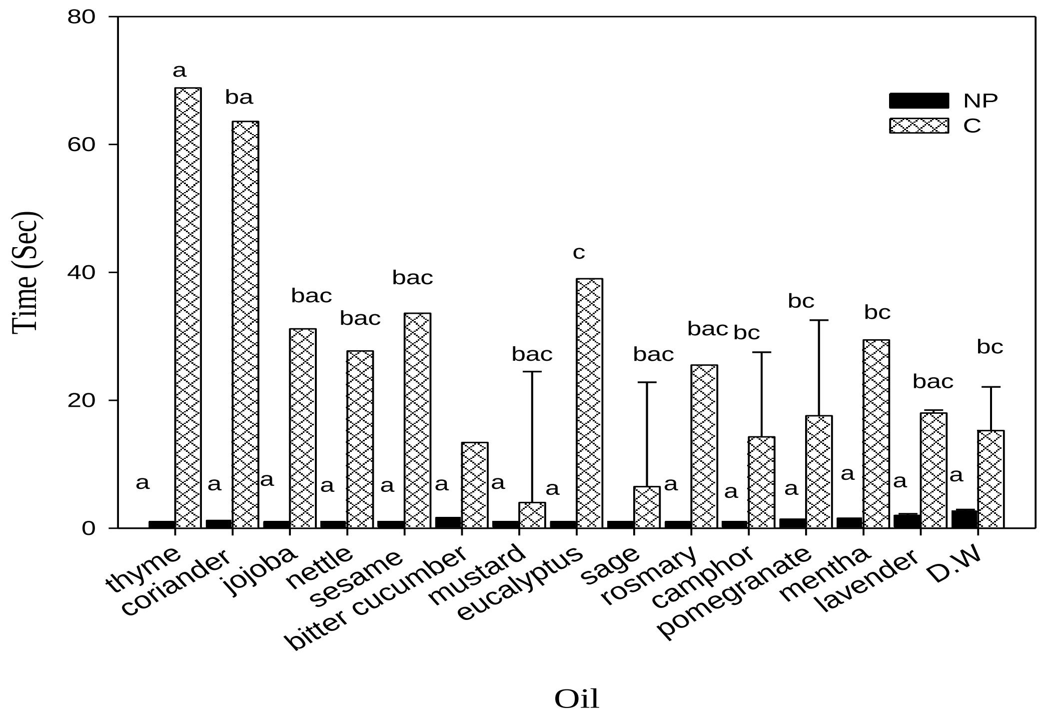

Figure 27: The Electrical Penetration Graph (EPG) was used to monitor the frequency of feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Figure 28 showed the results of E1 (associated with sieve element salivation or persistent virus inoculation) treatments had no significant difference compared with control. E2 (associated with sieve element sap removal). There was a significant between different plants treated, with EO and control(1 sec)., Jojoba, Sesame, Coriander, and Sage (8,3,2, and 2 sec., respectively Figure28). Bitter cucumber, Mustard, Eucalyptus, Camphor, Mentha, and Lavender, prevented GPA sieve element behavior for both E1 and E2.

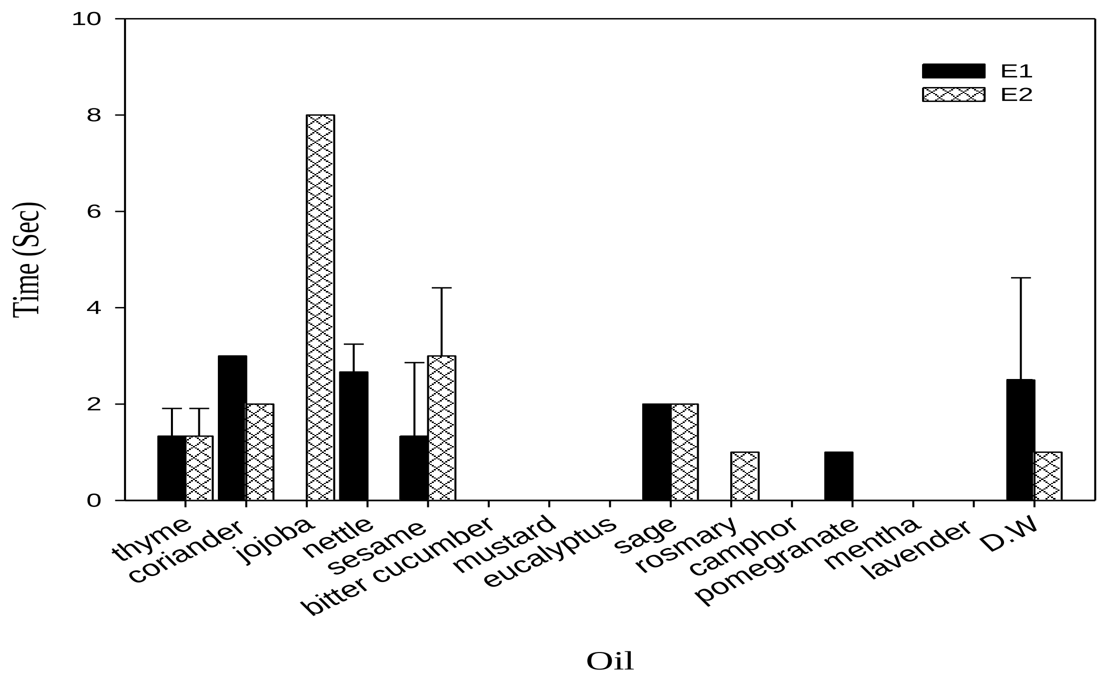

Figure 28: The Electrical Penetration Graph (EPG) was used to monitor the frequency of feeding behaviors E1 and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001. The absence of identical characters due to the absence of duplicates.

During the first two hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 29).

In (G) waveform a non-significant impact of Sage and Sesame (4.6 and 4sec) compared with control 3 sec. Mustard, Eucalyptus, Pomegranate, and Lavender did not show any result when they are compared with water control (Figure 29.A).

EO treatments showed (Figure 29.B) a non-significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Lavender, Mentha, Nettle, Jojoba, and, Pomegranate (59, 29,27,24, and 24 sec., respectively).

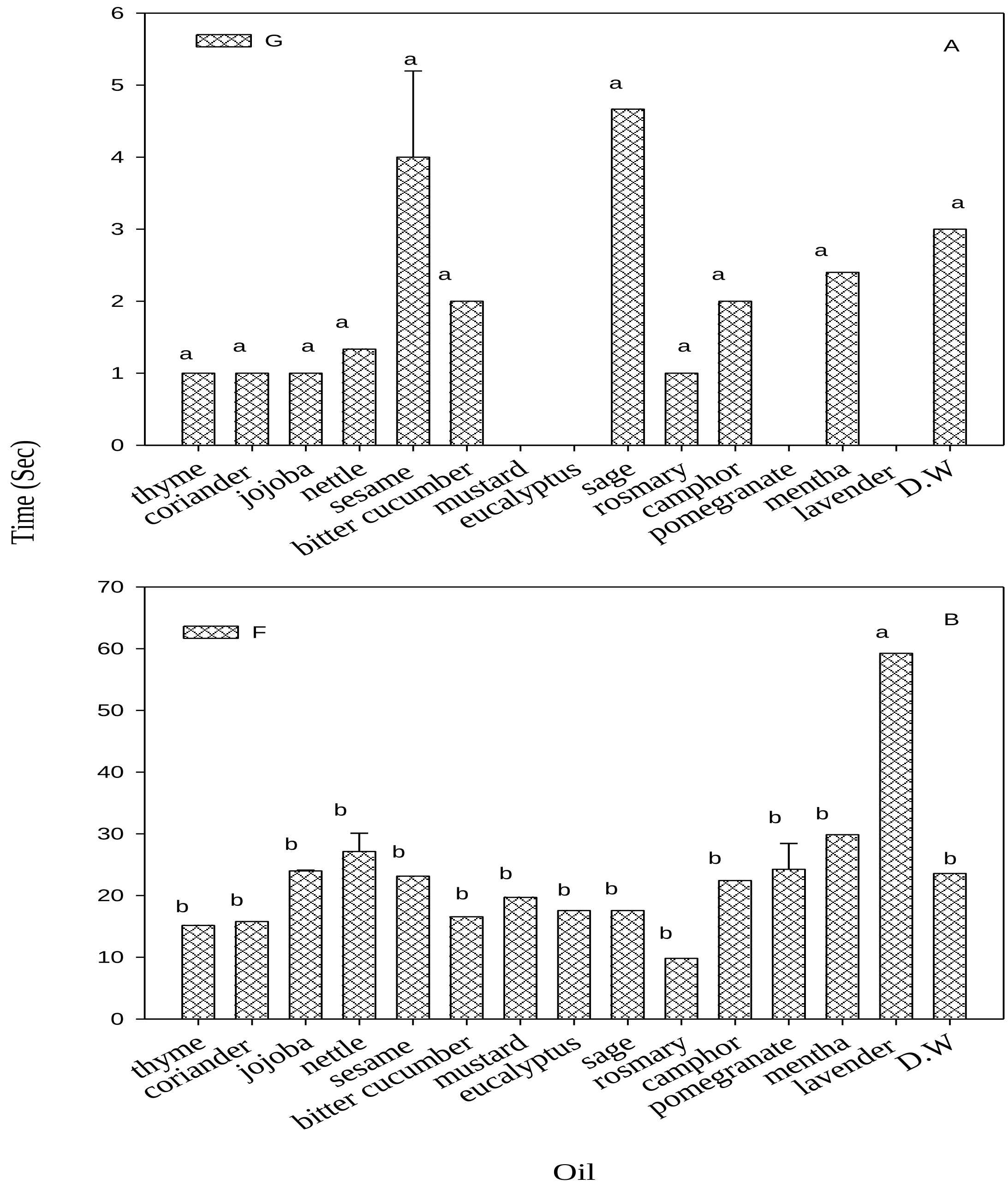

Figure 29: The Electrical Penetration Graph (EPG) was used to monitor the frequency of feeding behaviors G (A) and F (B) waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 30).

The electrical penetration graph record during the first two hours showed that EO treatments have significant impact on PD (figure 30) lavender oil recorded the highest reading of (62 sec.) compared with the control (35sec.) followed by Lavender, Coriander, Mentha, Nettle, Jojoba, and Pomegranate (54, 53, 47, 45, 41, and39 sec., respectively).

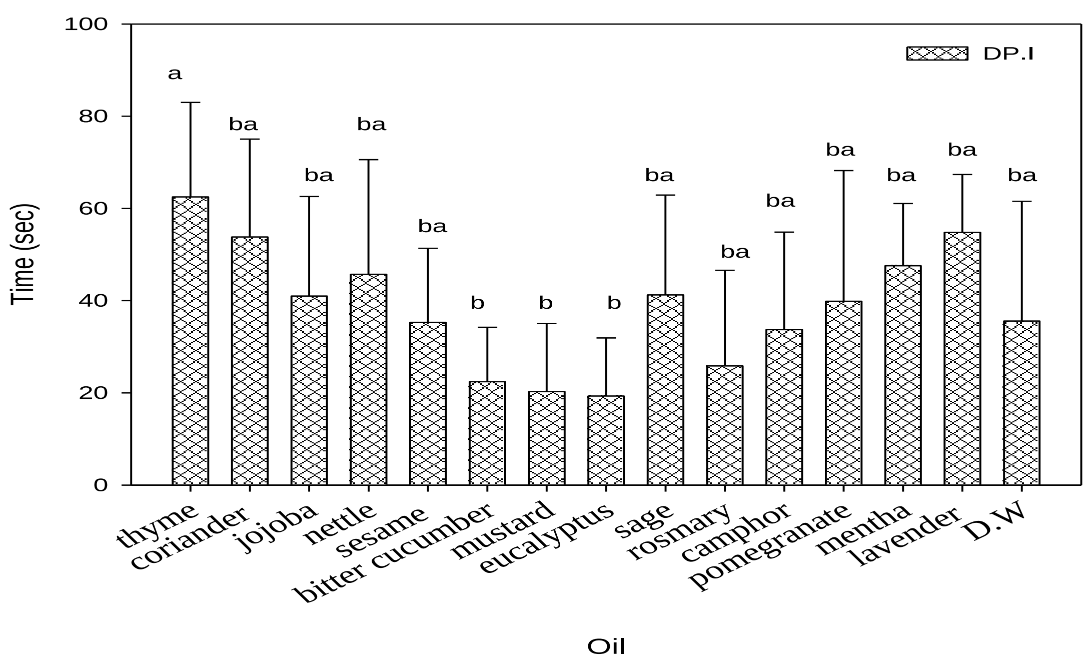

Figure 30 :The Electrical Penetration Graph (EPG) was used to monitor the frequency of feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001

4.4.6 The duration of Aphids waveforms on eight-hour recording

Figure 31 illustrated that during the eight-hour EPG recordings including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on plant treated with all EO compared with control. Although Coriander, Mustard, Sage, Jojoba, Thyme, Sesame, and Nettle during the pathway behavior (11065, 9306, 8390, 7727, 5439, 5287, and 3566 sec., respectively) compared with control (3380 sec) but was not significantly different.

Non-probing waveforms (NP), Nettle, Mustard, Rosemary, Mentha, and Bitter Cucumber recorded the longest delay in GPA settling behavior (6268, 5508, 3392, 2929, and 2340 sec., respectively) compared with control.

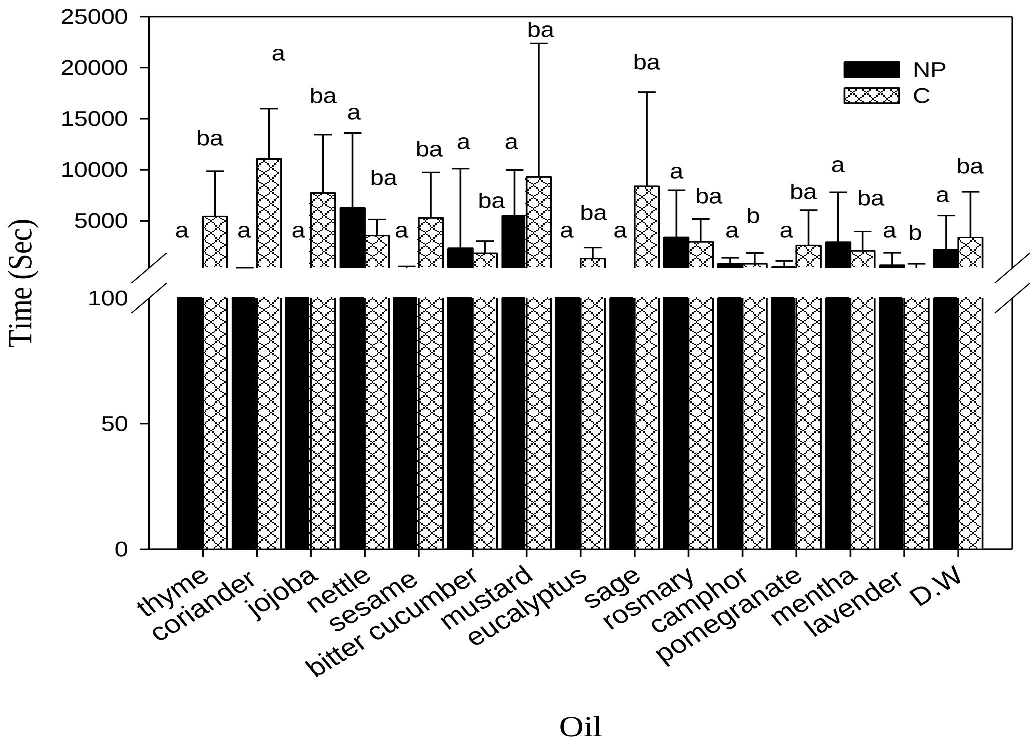

Figure 31: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001

Figure 32 showed the result of E1 (associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a non-significant delay between plants treated with EO and the control. Mustard, Eucalyptus, and lavender, prevented GPA sieve element behavior for both E1 and E2.

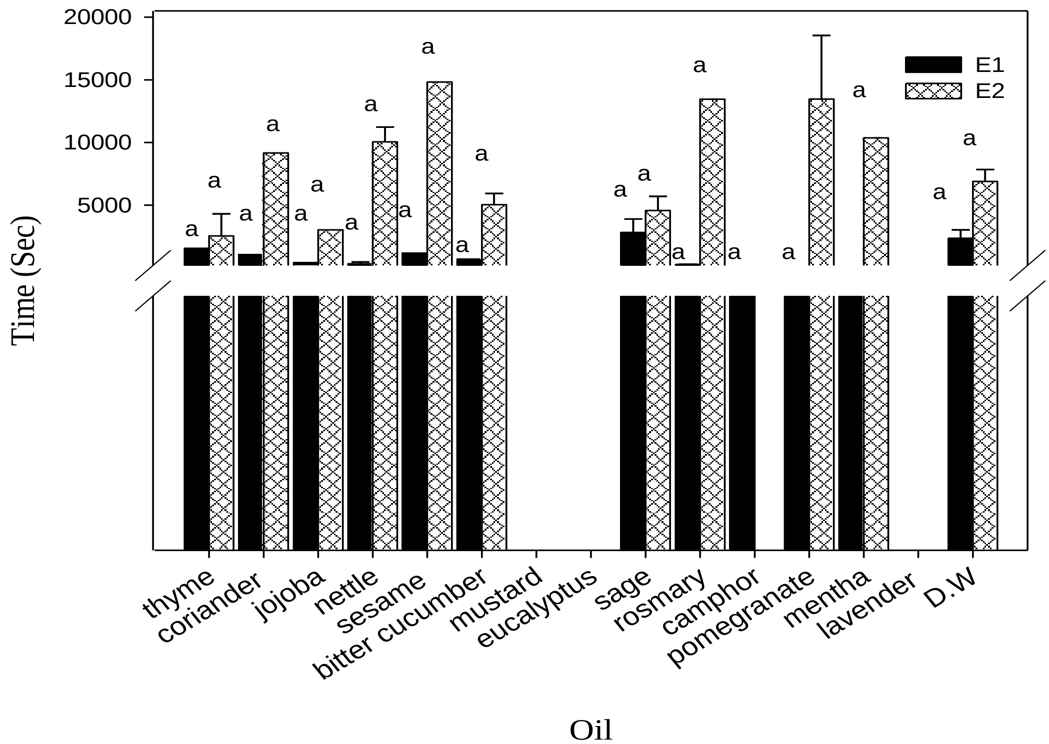

Figure 32: The Electrical Penetration Graph (EPG) was used to monitor the duration of feeding behaviors E1and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

During the first eight hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 33). In (G) waveform significant impact of Pomegranate, Sesame, sage, and Mentha (323, 37, 30, and 29 sec., respectively) compared with control (23 sec.) (Figure 33.A) However, Mustard, Eucalyptus, and Lavender prevented GPA xylem feeding behavior (Figure 33.A) EO treatments showed a non-significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Eucalyptus, Camphor, and Lavender (27789, 27415, and 27103sec).

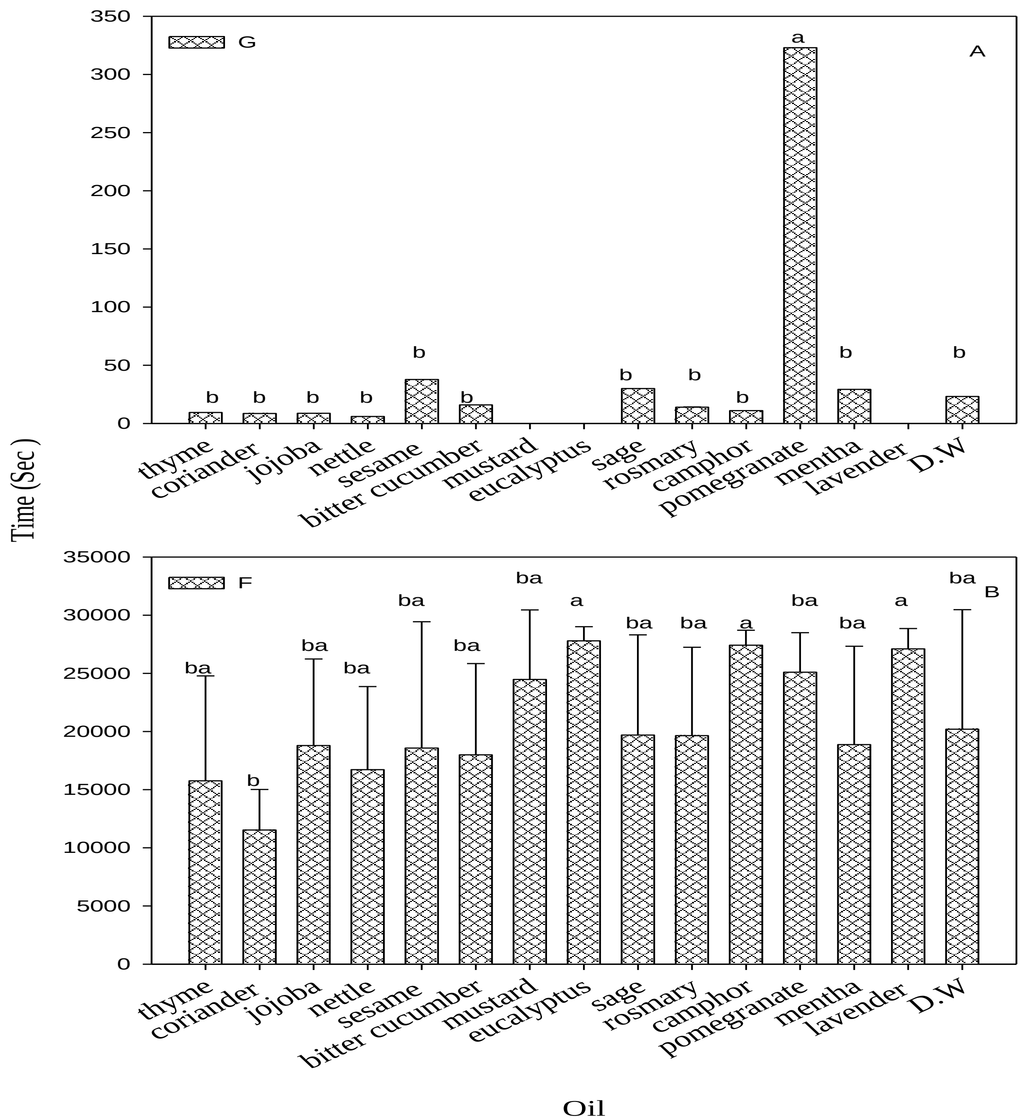

Figure 33: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors G(A) and F(B) waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 34).

The electrical penetration graph record during the first eight hours showed that EO treatments have a significant impact on PD (figure 34) Coriander oil recorded the highest reading (766 sec.) compared with the control (476sec.) followed by Lavender, Sesame, Jojoba, Sage, Thyme, and Pomegranate (727, 627, 569, 546, 490, 484 sec., respectively).

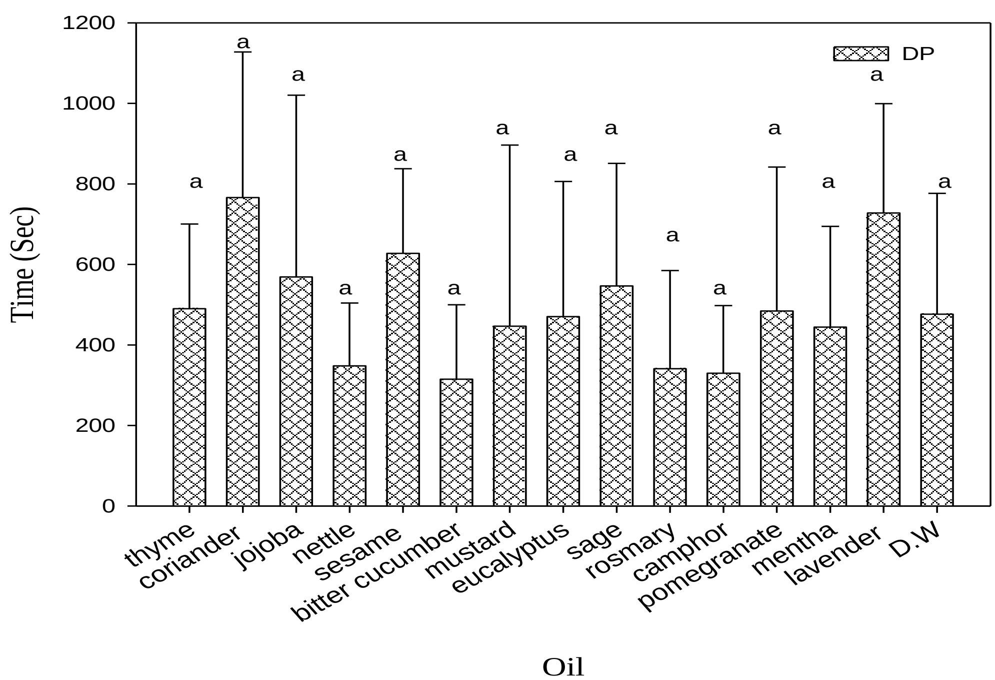

Figure 34: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

4.4.7 The frequency of Aphids waveforms on eight-hour recording

Figure 35 illustrated that during the first eight hour EPG recordings including pathway and non-probing behavior. In pathway waveform (C) reported no significant impact on plants treated with all EO compared with control. Although Coriander, Thyme, Jojoba, Sage and, Sesame during the pathway behavior (223, 145, 102, 97, and 83 sec., respectively) compared with controls (69sec) but was not significantly different.

non-probing waveforms (NP), no difference between all oil compared with control.

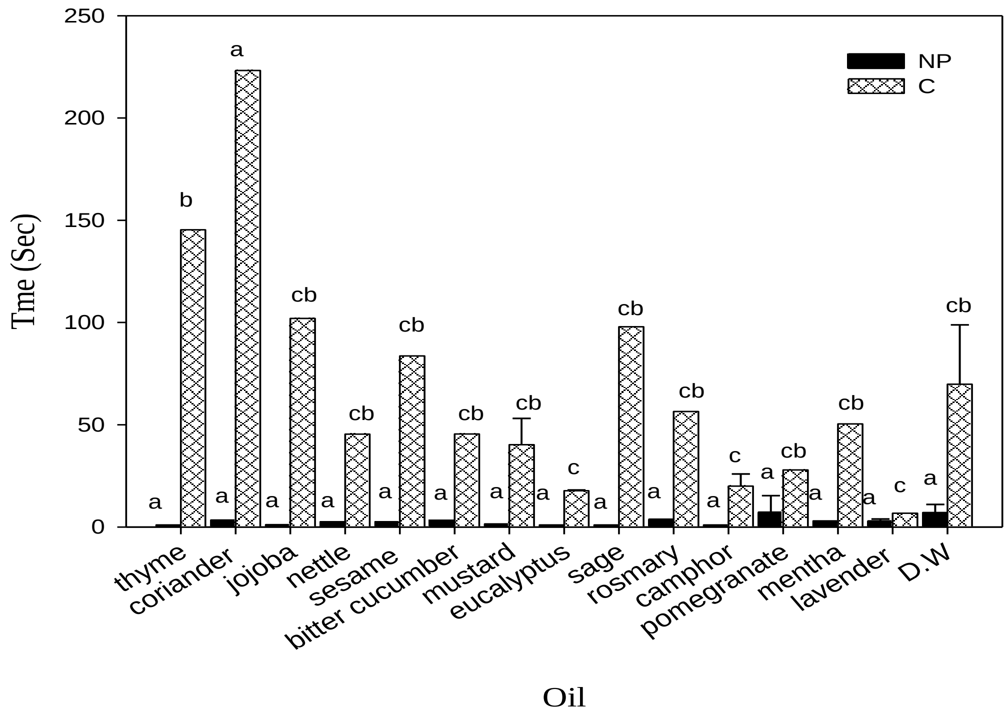

Figure 35: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors NP and C waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Figure 36 showed results of E1 (associated with sieve element salivation or persistent virus inoculation) and E2 (associated with sieve element sap removal). There was a non-significant delay between plants treated with EO and control. Thyme, Coriander, Sesame, Sage, and Rosemary (9.6, 7.2, 5.7, 4.5, and 4.5 sec., respectively (Figure 36), While in (E2) sap removal (E2) EO treatments had no significant difference compared with control (Figure

36). Mustard, Eucalyptus, and Lavender, prevented GPA sieve element behavior for both E1 and E2.

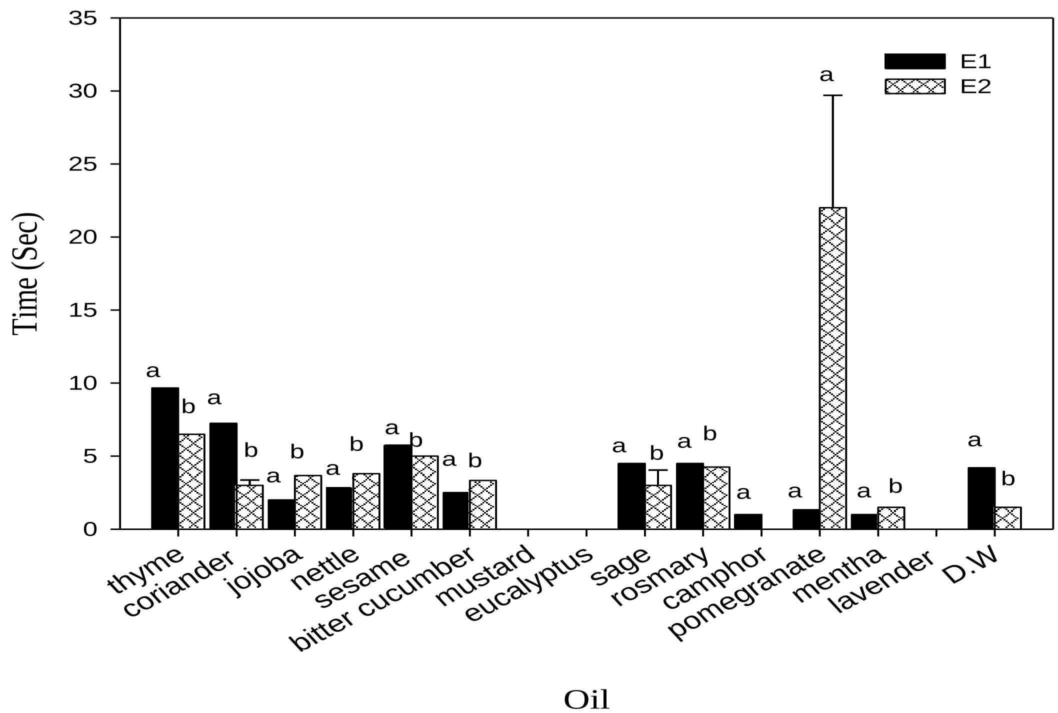

Figure 36: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors E1 and E2 waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P=0.001

During the first eight hours, recording GPA xylem feeding (G) and difficulty of penetration and the feeding dealing (F) have been shown in (Figure 37). In (G) waveform a non-significant impact of Pomegranate, Sesame, Sage, Mentha (323, 37, 30, and 29 sec., respectively) compared with control (23 sec.) (Figure 37.A). However, Mustard, Eucalyptus, and Lavender prevented

GPA xylem feeding behavior (Figure 37.A) EO treatments showed a significant impact compared with control on GPA penetration difficulty and the feeding dealing (F). Lavender, Sesame, Pomegranate, Eucalyptus, Mustard, and Jojoba (191, 91, 89, 82, 76, and 70 sec., respectively Figure 37.B).

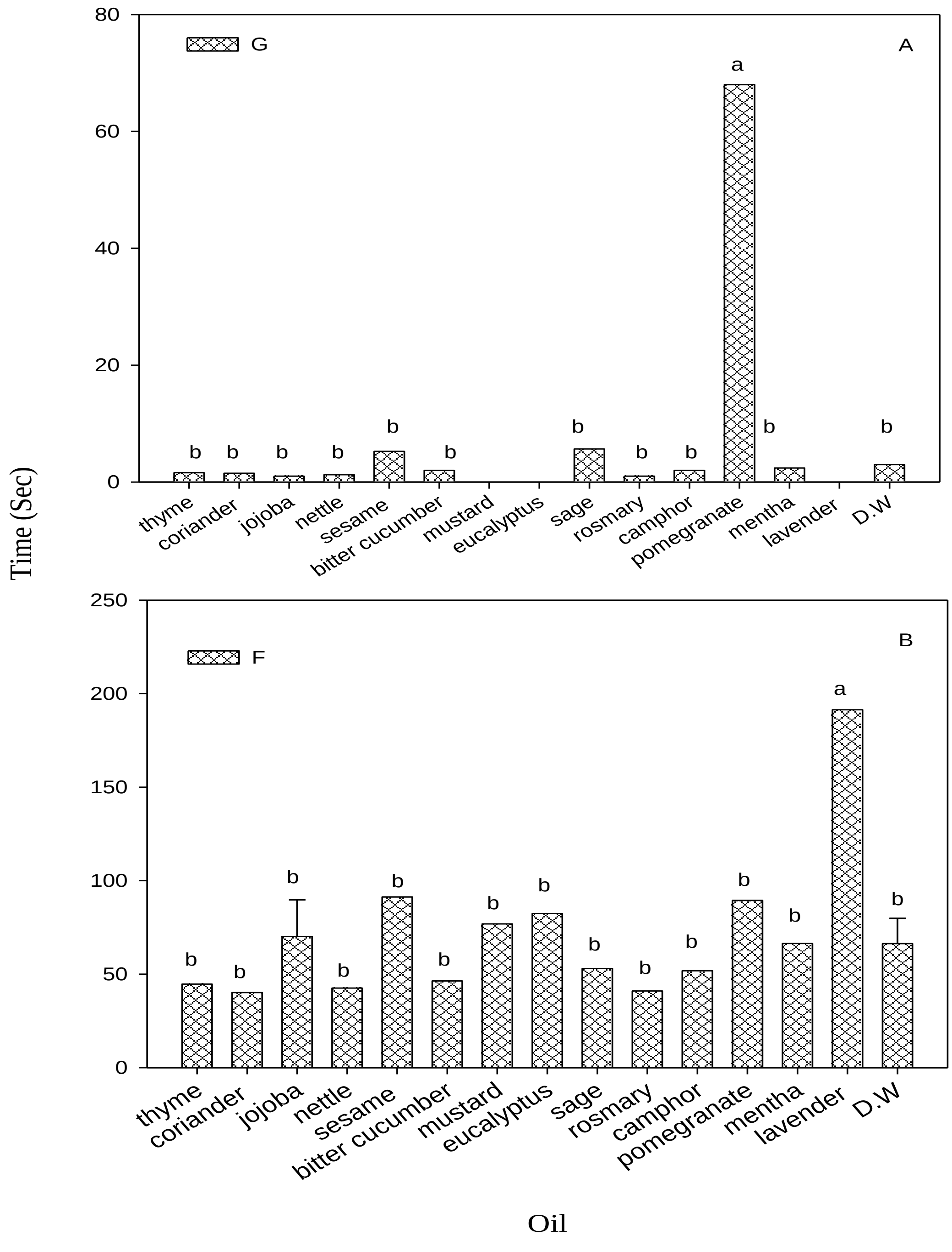

Figure 37: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors G(A) and F (B) waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

Potential drop GPA behavior related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses (PD) results shown in (Figure 38).

The electrical penetration graph record during the first two hours showed that EO treatments have a significant impact on PD (Figure 38). Coriander oil recorded the highest reading (195 sec.) compared with the control (112sec.) followed by Lavender, Sage, Thyme, Jojoba, and sesame (118, 115, 136, 135, and 133 sec., respectively).

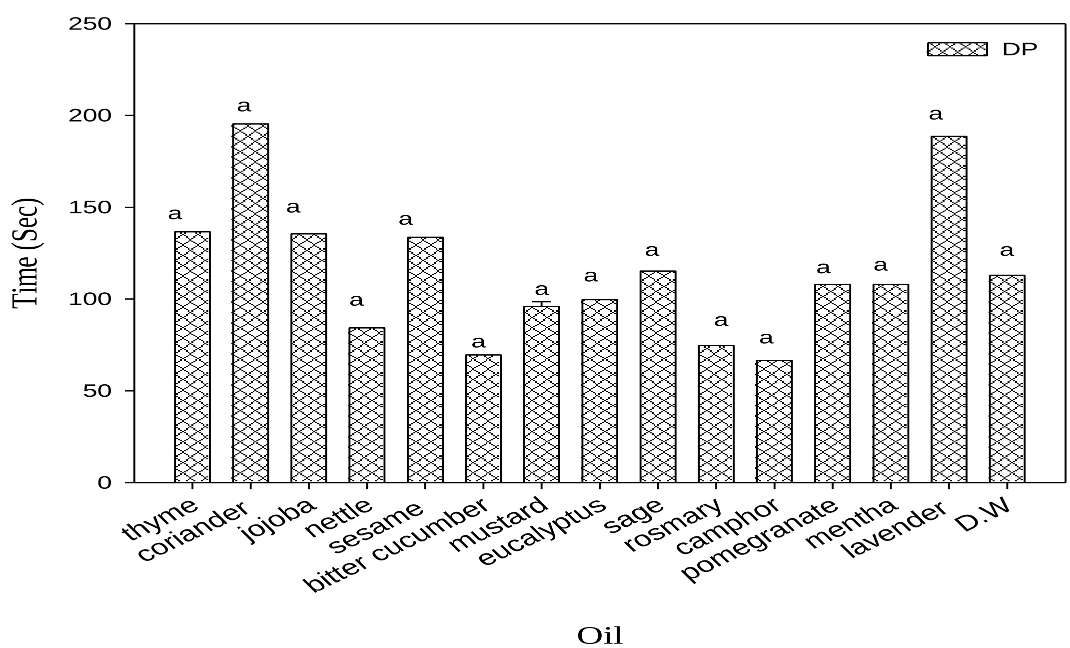

Figure 38: The Electrical Penetration Graph (EPG) was used to monitor feeding behaviors DP waveform in potato plant after EO treatment, results represent mean and Std of 8 replicates. Similar letters represent the same impact at P= 0.001.

5 Discussion

Potato aphid is one of the most harmful insect pests of fruits, vegetables, and ornamentals worldwide, The long-term applications of chemical insecticides have resulted in high residue accumulation in the different environmental components. They harm non-target organisms, ecosystems, and human health. Therefore, essential oils (EOs) from plant origins could replace conventional insecticides (Isman, 2000).

Essential oil activity against aphid carried out in-vitro in this study showed that a high percentage of EO increased plant dry/ fresh weight, higher plant growth rate, and higher nutrient content. In addition to its insecticidal impact on aphid. Results revealed that lavender and thyme were highly toxic to aphids. This could be because both oils contained high percentages of linalool, and thymol (Danh et al., 2013). Linalool; can inhibit acetylcholinesterase in insect pests (López & Pascual-Villalobos, 2010). *T. vulgaris* EO contained high amounts of thymol, a monoterpene phenol that binds to post-synaptic GABA receptors associated with chloride channels in insects (Priestley et al., 2003). When directly applied to aphids, the LC_{50} values of lavender and Thyme were very similar to those of linalool and thymol, which suggested that the insecticidal activity of the EOs was mainly due to their primary constituents. Oil of coriander (*C. sativum* L.), lavender

(*L. spica* L.), fennel (*F. vulgare* Mill.), oregano (*O. vulgare* L.), juniper (*J. communis* L.) and clove (*S. aromaticum* (L.) have shown strong insecticidal activity against cotton aphid within 24 hours after treatment (Ebrahimi et al., 2013).

Direct and indirect mechanisms of plant resistant pathways were used to suppress diseases and increase plant defenses against pathogens (Shabana et al., 2017). Promoting induced resistance in plant sequel response to pathogen attack, insect pest, rhizomicrobes, or by treatment with chemical or physical agents (Harun-Or-Rashid & Chung, 2017). Previous studies showed that plants can activate many different signaling pathways that depending on the types of infection (Walling, 2000). Resistance induction in plants was associated with variable antimicrobial compounds such as oxidoreductive enzymes like POX, PPO, and others (Kolattukudy et al., 1992). However, the possible functions of peroxidases in plant resistant mechanisms remain unclear (Datta et al., 1999). But several other studies documented the possible function that refers to POX and PPO. Oxidative enzymes have been shown to be associated in oxidize phenolics and other metabolites and it plays a key role in lignification of plant cells among defense mechanisms, also it has the ability for H_2O_2 degradation in plant cells (van Loon et al., 1998). It could be concluded that the elicitor character may play a crucial role in the efficacy performance of inducing resistance in plants. The current data obtained agreed with the previous studies in which organic compound (VOC)-mediated induced resistance against the sucking insect aphid, *M. persicae*; which had differences in their capability to activate induce resistance in the same host plant (Song & Ryu, 2013). Others reported that regulatory mechanisms and pathways among PPO and POX are different and it depend on the interaction between elicitors and host plants (Seo et al., 2008).

During EPG recordings including pathway waveform (c), Pomegranate and Sage recorded the longest delay in GPA settling behavior. In addition to non-probing (np). Camphor, Jojoba, and Sage recorded the longest delay in potential drop (PD) settling behavior and delay transfer of any potential of non-persistent and semi-persistent viruses. The longest delay was recorded for Sesame and Sage during E1 (associated with sieve element salivation or persistent virus inoculation), and Rosemary and Sage during E2 (associated with sieve element sap removal). Xylem feeding (G) has the longest delay on Jojoba and Sage, while the difficulty of penetrating plant by aphid and the feeding delaying (F) was recorded on Mustard and Sage. Mustard created stylet puncture in all living cells related to virus ingestion, inoculation, and acquisition. The result of the first time activities showed that Sage, Jojoba, and Mustard have a good result for the longest delay in aphid settling behavior and potential viral transmission.

Records of second-time behavior during the first two hours of the EPG recordings, pathway waveform (C) of Bitter cucumber, Sage caused the longest delay in the settling behavior of the aphid. In addition to non-probing (np), Bitter cucumber and Mentha recorded the longest delay in potential drop (PD) settling behavior and delay of non-persistent and semi-persistent viruses transmission. Results of E1 and E2, Sesame, and Sage have the highest recording time. Xylem feeding (G) has the highest on Bitter cucumber and coriander, and difficulty of penetration (F) was on Coriander and Mustard. It was found that Mustard and Eucalyptus created stylet puncture in all living cells that's courses virus ingestion, inoculation, and acquisition.

During the third time in the first two hour EPG recordings including pathway waveform (C) Eucalyptus, Sage recorded the longest delay in settling behavior. In addition to non-probing (np), the Bitter cucumber and Lavender recorded the longest delay in settling behavior and delay transfer any form of a virus. Results of E1 (associated with sieve element salivation or persistent virus inoculation) have the highest reading in coriander and E2 (associated with sieve element sap removal), have the highest for Jojoba.

Xylem feeding (G) has the highest recording in Mentha and Sage and difficulty of penetration plant by aphid and the feeding delaying (F) caused by Mustard and Sage. Both oils created stylet puncture in all living cells related to virus ingestion, inoculation, and acquisition. Duration and frequency during the first two-hour showed that Eucalyptus, have a low stylet puncture of non-persistent virus and plant damage. While duration and frequency during the eight-hour showed that Biter cucumber, Camphor, Nettle, and Rosemary, have a low stylet puncture of non-persistent virus and plant damage.

The current study described the EPG waveforms of aphid feeding and settling behavior. The time is taken by aphids to commence phloem-feeding (E2) was significantly different between plants treated with the different essential oils. The greater the E1/E2 salivation by viruliferous vectors on the plant will promote virus inoculation, thus the probability of transmission increased as a function of time spent salivating into the phloem. Moreover, transmission occurred as quickly as 5 min for the non-persistent virus. Previous studies have reported increased salivation by *R. padi* before sustained sap ingestion on nitrogen-deficient barley (Ponder et al., 2001), and by *M. persicae* on mutant Arabidopsis plants which had reduced levels of amino acids (Hunt et al., 2010); thus suggesting that salvation is an important part of improving plant nutritional quality for aphids. It is also known that aphid saliva contains proteins designed to overcome plant defenses such as forisome- and callose sealing of sieve tubes (Will et al., 2009). Furthermore, the PD frequency increased during the stylet pathway phase in plants treated with essential oil, despite the increase of the duration of the pathway. PD represent puncturing of cells along the stylet pathway; as the stylet breaches the plasma membrane placing the gold wire within the silver droplet on the insect’s abdomen in a manner that will not constrain the insect movements. Failing to perform those steps correctly will result in poor electrical connectivity, which will result in data of poor or unacceptable quality as well as difficulty feeding delay.

6 Conclusion and recommendation

This study investigated the role of medicinal plant oils in inducing potato resistance systems against aphid pest. In-order to alternate aphid chemical control with insecticides. This opens a wide door to the use of essential oil as an environmentally friendly method against aphids. It suggested using high numbers of oil type to confirm the ability of essential oil to induce plant resistance. Such essential oils could interfere with the settling and feeding behavior of sap-feeding insects such as aphids. Delaying in the first encounters to plant tissues would enhance the loss of non-persistent virus found in aphid mouth part before being injected in healthy plant tissue. Prolonging the searching behavior and detaining aphid from reaching phloem tissues would also enhance losing the semi-persistent and persistent viruses injected by viruliferous aphids.

Abstract in Arabic (الملخص)

تحفيز المقاومة الطبيعية لمحصول البطاطا باستخدام الزيوت العطرية النباتية كمحفزات لمكافحة منّ الدراق الذهبي ***Myzus persicae*** **(Sulzer) (Homoptera: Aphididae)**

الطالبة: رؤيا ابو الفيه

المشرفة: د. رنا سمارة

الملخص

يعد محصول البطاطا واحداً من أهم المحاصيل الزراعية المُنتجة بالعالم، يتعرض المحصول للإصابة بالعديد من الآفات الحشرية التي تسبب الكثير من الخسائر الاقتصادية .

تعتبر حشره مَن الدراق الذهبي *Myzuspersicae* (Sulzer) (Homoptera: Aphididae) من أهم الآفات الحشرية الاقتصادية على البطاطا، وتعتبر ناقل للعديد من الامراض الفيروسية النباتية. تعتمد مكافحة هذه الحشرة بشكل اساسي على المكافحة الكيميائية باستخدام المبيدات الحشرية، والتي يتم العمل على استبدالها بطرق أكثر أماناً ومناسبة للإنتاج الزراعي المستدام والأمن الغذائي.

في هذه الدراسة تم اختبار العديد من مستخلصات النباتات العطرية الفلسطينية لمعرفة مدى فعاليتها في تحفيز مسارات مقاومة النباتات العائل للحشرات، وذلك من خلال قياس مستوى النشاط الإنزيمي للبروتينات، وسلوك الحشرة.

اوضحت النتائج أنَ 60% من الزيوت العطرية النباتية المستخدمة في هذه الدراسة ذات فعالية ضد حشرة منّ الدراق الذهبي ، كما أظهرت النتائج أن بعض الزيوت النباتية كان لها تأثير على تحفيز

نشاط الإنزيمات المرتبطة بمقاومة النباتات للإصابات الحشرية و أثرت على سلوك الحشرة مقارنة مع عينة المرجع. حيث إن زيوت (الكافور، الميرامية، الرمان و الحنظل) أحدثت اضطراب في سلوك الحشرة بالإضافة إلي إطالة فترة البحث عن موقع التغذية، بينما قام كل من زيت (الخردل، الكينا و الميرامية) بتأخير نقل الفيروسات الغير مستمرة و شبه المستمرة، وأحدث زيت (الخزامي، الكينا و الخردل) تأخير انتقال الفيروسات المستمرة (الدائمة).

هذه الدراسة تمكنت من التحقق من امتلاك الزيوت العطرية النباتية دور مهم وفعال في تحفيز المقاومة الداخلية للنباتات. وبالتالي فهذه النتائج تفتح المجال لتقليل استخدام المبيدات الكيميائية لوقاية النباتات.

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