



Chemical composition and Morphological Characters In Fences Plants As A New Direction In Resistance Against *Tetranychus Urticae* Koch



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Abstract

Chemical composition and morphological characters Fences plants are the first defense soldiers against many pests also against *Tetranychus urticae* (Acari: Tetranychidae), so this study has been done to confirm that the aromatic plants are the first defense soldiers and compose infesting of pests for plants growing beside it. Two replicates (with zero and 10 % infestation with *T.urticae*) per each *Lantana Camara* and *Dodonaea Dodonaea* potted plants were used to evaluate the changes before and after infestation to their compounds. After two weeks of growing, the plants cut and dried. Cinnamic (7.97%) and kaempferol (37.65%) after infestation with *T. urticae* as the major compound in *l. camara*, while *D. Dodonaea* contains Apigenin-7-glucoside (3.19%) and Caffeic (0,67%). The most founded in Phenolic acids was Rosmarinic acid 12.43 after infestation but *D. Dodonaea* 23.43. also Sinapic acid 87 and 23 Coumarin acid 43.21 Protochatchuic 17,2 Rutin acid 43,21.1 Syngic acids 54,0 for *L. Camara* and but *D. Dodonaea* respectively as the most abundant component after infestation. *L. Camara* dried leaves were covered with gold utilizing an Edwards Scancoat SIX sputter-coater and thusly analyzed in a Phillips XL 30 ESEM magnifying lens utilize the HiVac modus (i.e. auxiliary electron identifier, increasing speed voltage 15Kv). Ten grams of the samples were put in water distillation (500 ml water) using a Clevenger's apparatus. The distillation was continued for 4 h after boiling. The volatile substances were isolated and dried over anhydrous Na₂SO₄. The most of planting as fences plants for their morphological and phytochemical studies that's makes it more effective for planting grows.

Keywords : Chemical Composition, Morphological Characters, *Tetranychus Urticae*, Organic Farming, *Lantana camara*, *Dodonaea Dodonaea*

1. Introduction

Plant species have high-level international flexibility to protect themselves in reaction to herbivores' assault. Direct methods involve morphological features like hairs, trichomes, compositions like latex, acyl-sugars, and waxes, which are the initial barrier to deter insect attacks. [1] Plant species also manufacture defense proteins and tiny compounds eaten by pests, that repel or toxic and impair survival. They can also be used in herbivorous vegetation. [2,3] plant life and pesticides beside each other for over 350 million years In co-evolution, both have evolved strategies to avoid each other's defense systems. Natural pest protection not only requires the secondary build-up of metabolites, as well as on

hormone signaling. Mostly Salicylic Acid (SA), Jasmonic Acid. [4] Are regulated by various phytohormone signal transduction. This development between plants and pests has resulted in a gorgeous defense system in plants that can be familiar with the no-character molecules or signals from smashed cells, much like the animals, and activates the plant immune response against the herbivores [5,6] To counter the herbivore do violence, plants fabricate specialized morphological structures or secondary metabolites and proteins that have toxic, repellent, and/or anti-nutritional special effects on the herbivores. [7,8] Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface

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of the plants e.g., hairs, trichomes, spine, and thicker leaves, or manufacture of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either destroy or retard the development of the herbivores. [9] meandering defenses against insects are mediated by the release of a blend of volatiles that specifically attracts natural enemies of the herbivores and/or by providing food (e.g., extrafloral nectar) and housing to improve the efficiency of the natural enemies. [10] Plants respond to herbivore attacks through a complex and active defense system that includes structural barriers, toxic chemicals, and the hold of natural enemies of the aims pests. [11] In cooperation security mechanisms (direct and or indirect) may be nearby constitutively or induced after damage by the herbivores. Induced reaction in plants is one of the significant components of insect control in cultivation and has been exploited for the guideline of insect herbivore populations. If induced response occurs very near the beginning, it is of great benefit to the plant and reduces the subsequent herbivore and pathogen harass, in addition to civilizing the overall condition of the plant. . [12] This study aims to find the most fences plants that make defense line against *T.urticea* by studying Chemical composition and Morphological Characters that make it more resistant for there and planting grows beside.

2. Experimental

2.1 Chemical Studies

GC/MS analysis of the acetone insoluble fraction, The fatty alcohols mixture was subjected to GC/MS analysis using the following conditions; Gas chromatography: Agilent 6890 gas chromatograph equipped with an Agilent mass spectrophotometer with a direct capillary interface and fused silica capillary, HP-5MS Phenyl Methyl Siloxane (30m x 250µm x 0.25µm film width). the solvent delay was 7min. and the injector size was 10.0µL. The mass spectral detector was operated in an electron impact mode with ionizing energy of 70 eV scanning from m/z 50 to 700. The ion source temperature was 230°C and the quadruple temperature was 150°C. The GC temperature program was started at 190°C (15min.), then elevated to 280°C at a rate of 4 °C/min. The detector and injector temperature was set at 280°C and 250°C respectively. Gas chromatographic analysis of unsaponifiable matters 1-Instrument, 6890 GC method, 2- Oven, Initial, temperature: 70°C.[13] Two replicates (with non and 10 % infestation with *T. urticae*) per each *L. Camara* and *D. Dodonaea* potted plants were used to assess the changes before and after infestation to their

compounds. After two weeks of agriculture, the plants cut and dried.

a. Extraction of plant substance in different parts

Ten grams of foliage were subjected to water refining (500 mL water) utilize Clevenger's mechanism. The refining has proceeded for 3 hours in the stir of bubbling. The random substances were secluded and dried over anhydrous Na₂SO₄ as per Guenther.

b. Identification of the concoction organization of oils

The parts of virtuous were recognized by GC/MS, utilizing GLC Hewlett Packard model (5890) arrangement II additionally, furnished with a carbo wax 20 M slim segment (0.32 mm x 50 m, i.d.), fire ionization indicator (FID), helium as bearer gas at a stream rate of 1 ml/min, starting segment temperature was 60°C expanded to 200°C at a rate of 3°C/min also, hold at 200°C for 40 min, injector, and identifier temperatures were 200 and 250°C, individually. MS examination was made utilizing the Hewlett Packard Mass Spectrometry model (5970). Temperature ionization locator (TIC) finder was utilized, Carbowax 20 M hair like section (0.32 mm x 50 m, i.d.), the temperature was expanded from 60 to 200°C by 3°C/min what's more, MS ionization voltage was 70 eV. Subjective distinguishing proof of the oil constituents was done by looking at their preservation times and mass fracture designs with those of the available authentic in the information with the script information.

2.2 Morphological studies

Dried-out leaves were sheltered with gold utilizing an Edwards Scancoat SIX sputter-coater and thusly analyzed in a Phillips XL 30 ESEM magnifying lens utilizing the HiVac modus. Anatomical components were broken down on the lower surface of the leaves. The kind of stomata, epidermal cells, glandular trichomes, non-glandular trichomes, and trichome thickness as well were recorded and the distance between the glandular trichomes was measured. For each of these subjective and quantitative characters, no less than 30 perceptions for every populace were made.[14,15,16] The same leaf pair concentrated morphologically was likewise utilized for anatomical investigation[17,18]. This part of the work was done with the assist of the Applied Centre of Entomonematodes, Faculty of Agriculture, Cairo University, Giza governorate.

3. Results and Discussion

3.1 Chemical Studies

This monoterpene is known for its effectiveness as an insect repellent .The present results revealed that *L. Camara* oils contain

Rosmarinic(3.63%), **Cinnamic** (7.97%), and **kaempferol**(37.65%) after infestation with *T. urticae* as the major compound in *L. camara*, while *D.*

Dodonaea contains **Apigenin-7-glucoside** (3.19%) and **Caffeic** (0.67%) as the most abundant component after infestation.

Fig.1. The molecular structure of the main components founded after infection in *L. Camara* and *D. Dodonaea* leaves

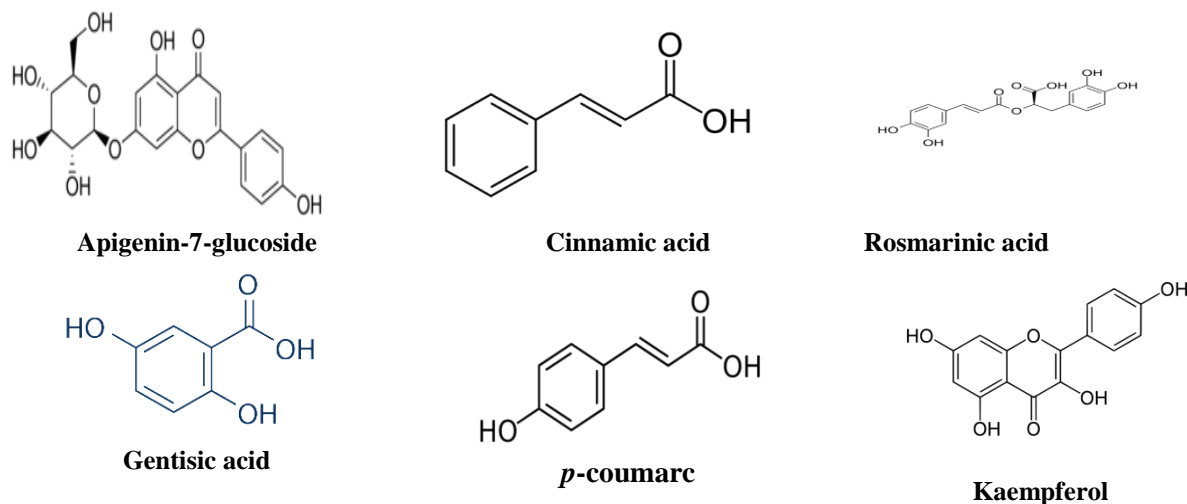


Table 1: High GC/MS chromatogram of volatile oil extracted from [$\mu\text{g. ml}^{-1}$ *L. Camara* and *D. Dodonaea* leaf before and after infesting of check plots as $\text{mg} \cdot \text{g}^{-1}$ of leaves.

Compound	Tr (min)	sample conc (mg/g extract)			
		<i>L. Camara</i> before	<i>L. Camara</i> after	<i>D. Dodonaea</i> before	<i>D. Dodonaea</i> after
Galic acid	3.9	0.58	0.00	22.63	6.57
Protocatechuic acid	7.65	0.00	0.00	0.43	0.00
p-hydroxybenzoic	11.88	0.00	0.00	0.00	0.00
Gentisic acid	11.8	0.00	0.74	2.06	1.09
Catechin acid	14.74	0.00	1.34	0.71	1.12
Chlorogenic acid	15.95	0.00	0.42	0.00	0.14
Caffeic acid	16.79	0.00	0.14	0.00	0.67
Syringic acid	18.67	0.00	0.84	0.00	0.00
Vanillic acid	20.55	0.00	0.00	0.06	0.00
Ferulic acid	28.27	0.00	0.00	0.20	0.00
Sinapic acid	30.156	0.00	0.18	1.47	0.96
p-coumaric acid	34.4	0.00	0.53	0.94	0.94
Rutin acid	33.98	0.00	0.00	4.22	2.90
Apigenin-7-glucoside	37.93	0.00	0.00	2.95	3.19
Rosmarinic acid	38.8	0.79	3.63	0.00	0.00
Cinnamic acid	46.12	0.61	7.97	0.00	0.00
Quercetin acid	48.82	0.00	0.68	0.00	0.00
Kaempferol acid	55.06	8.28	37.65	0.13	0.10
Chrysin acid	59.03	0.11	0.38	0.83	0.11

Secondary metabolites have been mainly studied as the mediators of direct defense,) however much is to be done to reveal the unidentified or emerging signaling pathways[19,20]. Mass spectrometry used for the derived metabolite profile and gene

expression examination by high-throughput sequencing has made this field more exciting and cost-effective. A study on secondary metabolites could lead to the identification of new signaling molecules involved in plant resistance against

herbivores [21,22,23] and other stresses this clear in fig 1 could be known. we recommended that growing

L.camara fences plants for biological besides mechanical role against defoliant pests.

Table 2: HPLC fractionation of phenolic acids extracted from *L. Camara* and *D.Dodonaea* leaves of check plots as mg/5g of leaves.

Compound	ug/ml			
	<i>L. Camera</i> before	<i>L. Camara</i> after	<i>D. Dodonaea</i> before	<i>D. Dodonaea</i> after
Chrysin acid	5.84	ND	1.73	ND
Rosmarinic acid	5.32	12.43	3.3	23.43
Kaempferol	0.1	3.7	3.4	2.1
Cinnamic acid	0.52	2.61	5.46	9.50
Quercetin	43	ND	3	1
Sinapic acid	43	87	5.1	23
Gentisic acid	13	22.32	3.8	ND
Coumarin acid	ND	43	1.2	21
Syrngic acid	32	54	2	ND
Caffeic acid	12	123	ND	21.1
Rutin acid	14.3	43	2.1	21.1
Protochatchuic	3	17	12	2
Ferulic	21	77	1	2.4

phenolic acids extracted from *L. Camara* for Rosmarinic acid 12.43 after infestation but. *Dodonaea* 23.43. also, Sinapic acid 87 and 23 Coumarin acid 43, 21 Protochatchuic 17, 2 Rutin acid 43, 21.1 Syrngic acid 54, 0 for *L. Camara* and but *D. Dodonaea* respectively Phenolics serve dual functions, of both repelling and attracting different organisms in plants' surroundings. They act as protective agents, inhibitors, natural animal toxicants, and pesticides against invading organisms, such as herbivores, nematodes, phytophagous insects, and fungal and bacterial pathogens

3.2 Morphological studies

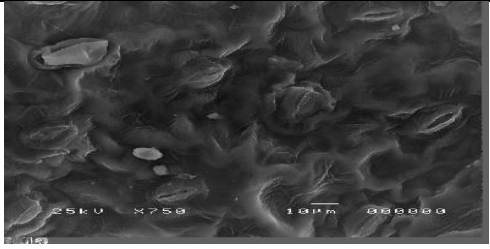
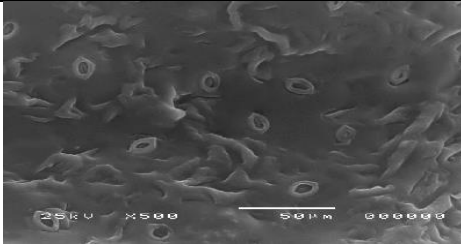
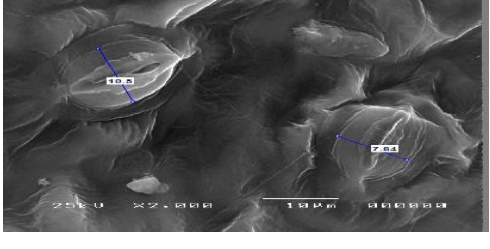
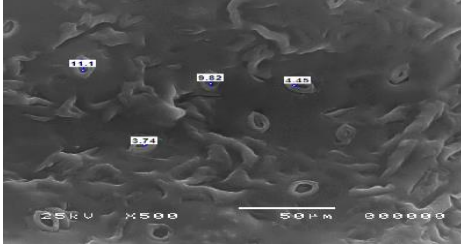
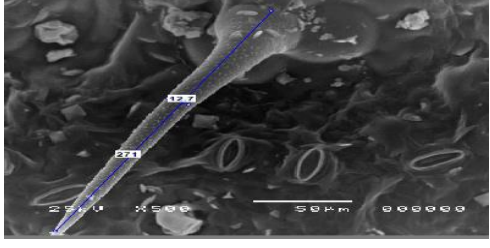
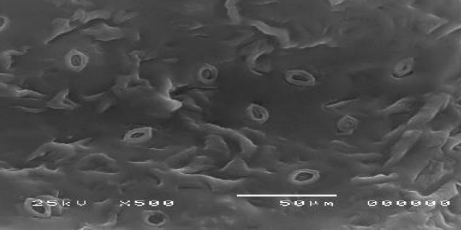
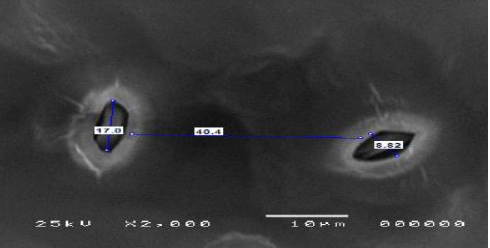
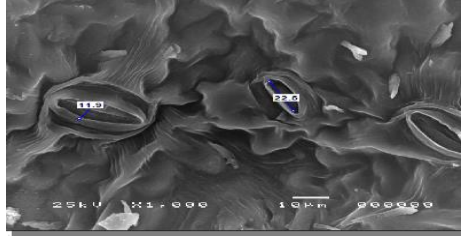
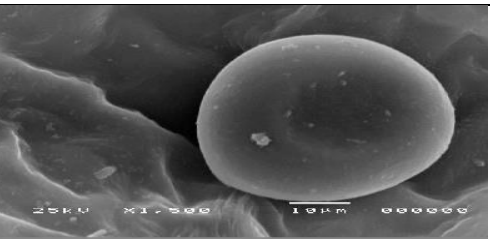
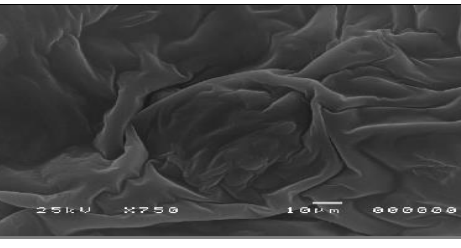
In medicinal plants, small and spiky non-glandular trichomes were dominantly appeared on the adaxial surface, whereas capitate glandular trichomes were commonly localized on the abaxial surface. In some medicinal plants, single non-glandular trichomes and peltate trichomes were found on the adaxial surface [24]. The number of respiratory stomata in *D. Dodonaea* was fewer than *L. Camara* in (16 & 18 per ml), respectively; respiratory stomata diameter was much similar in both (13.2 & 17.5), respectively; trichome length in was shorter than *D. Dodonaea* (0 & 27.2) respectively; the number of oil glands was (11 & 15), respectively in *D. Dodonaea* and *L. camara*; diameter of oil glands on upper and under surfaces in *L. Camara* (17 ml) was smaller than in *D. Dodonaea* (0), [25,26], respectively; the mites selected the same place on leaves (between oily glands, trichomes, and respiratory stomata) of both

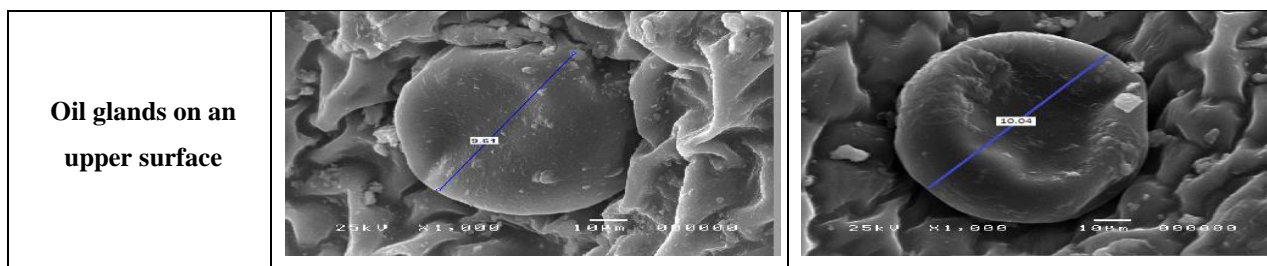
plants when feeding. Hair cover consisted of non-glandular and glandular hair types observed on the upper and lower leaf surface. [27,28] The non-glandular hair was unicellular or multi-cellular, some of which had cuticular small papillae, and referred to two types of hairs: un-branched and dendroid hairs. Un-branched trichomes were found on both surfaces of the leaf of almost all studied mints. In contrast, dendroid trichomes were observed on the abaxial leaf surface of few species only. These mints can accordingly be discriminated from all other species by possession of dendroid hairs. Based on the presence of the dendroid trichomes, [29,30,31,32] were defined to record the non-glandular hair cover. Most of these composite are likely products of glands, but some may be formed by epidermal cells and then out of sight through the cuticle as with surface waxes. In several studied cases, the response of microorganisms to exudates compounds have been compared in vivo and on plant tissue as in the case of the sesquiterpene lactone parthenolide from *Chrysanthemum parthenium* [33]. The morphological characters such as respiratory stomata, [34,35] trichomes, and oily gland (numbers and dimensions) help the plant leaves in mechanical defense against *T. urticae* which is reflected in population dynamics and biological control. Also, several flavonoids such as flavones, flavonols, flavan-3-ols, proanthocyanidins, flavanones, isoflavonoids, and flavans are not only feeding deterrents against many insect pests but also have antifungal activity. [36,37,38,39,40] reported the significant role of

flavonoids (quercetin and rutin) and simple phenol (ferulic acid and caffeic acid), were toxicity and repellence against insects. The impact of the attack by mites was observed on the content of all phenolic compounds found in comparison with the control ones (two species of mint). Almost all of the leaves

attacked by mites had greater amounts of all the phenolic acids by HPLC. Greater amounts of phenolic acids were found in the infected mint species. Thus, it may be concluded that its induction had a crucial role in the defense strategy against the mite.

Table 3: Scanning Electron Microscopy comparison between *L. Camara* and *D. Dodonaea* leaves structures.

Structures	<i>lantana camara</i>	<i>D. Dodonaea</i>
No. of respiratory stomata		
Respiratory stomata diameter		
Trichomes length		
Respiratory stomata diameter		
Oil glands under surface diameter		



Morphological structures. Plant structures are the first line of defense against herbivory and play an important role in HPR to insects. In **table 3** shows The earliest line of plant defense against pests is the erection of a physical barrier either through the arrangement of a waxy cuticle,9,16, and/or the development of spine, setae, and trichomes.18,19 Structural defenses include morphological and anatomical traits that confer a fitness advantage to the plant by directly deterring the herbivores from feeding,16 and range from prominent protuberances on a plant to microscopic changes in cell wall thickness as a result of lignification and

suberization.9,19 Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) take part in a leading role in plant protection against herbivory. Sclerophylly refers to the hardened leaves and acting a dynamic position in plant defense against herbivores by dropping the palatability and digestibility of the tissues, thereby, reducing the herbivore harm.

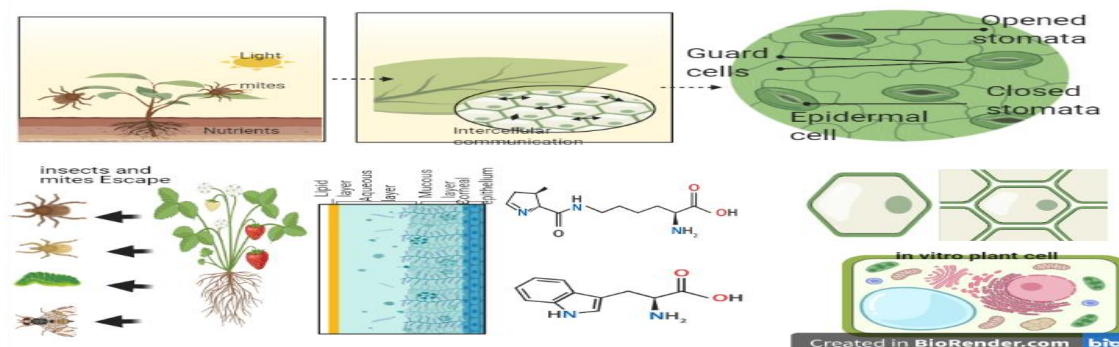


Fig.2.Mechanism of induced resistance in plants against different pests.

In **fig. 2.** showed the mechanism of induced resistance in plants against different pests, While numerous genes that participate in the synthesis and management of vapors in plants are known this is in agreement with [41,42]. beforehand reported being induced in reaction to *T. urticae* infestation [43]. TSSM populations evaluated the transcriptome and chemical characteristics under both outdoor and temperature control conditions. Various research comparing genotypes for the re-introduction into cultivated lines of favorable characteristics such as VOC emissions from wild species is parallel with [44,45,46]. Arthropod tolerance is usually not a necessity [47,48] with this study and other studies we will be able to avoid resistance against *T. urticae*. [52]Nevertheless, there's considerable genetic diversity for plant protection from insects, as may be demonstrated by contrasting wild and

domesticated genotypes resistance for other arthropods [49,50,51].

4. Conclusions

The morphological studies show that the number of respiratory stomata and oil glands in the *L. camera* was more than in *D. Dodonaea*. Also, the trichomes were longer. Histological studies showed that the epidermis of *L. Camara* had a greater presence in the number of mites than in *D.Dodonaea*. Phytochemical studies found that more compounds lead to the process of resistance and expulsion of pests in *L.Camara* after infestation than in *D.dodonaea*. Finally, from these studies, it can be said that *L.Camara* resisted *T. urticae* infestation by increasing their secondary metabolites which are considered to be the plant defense.

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