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Comparative Morphology and Chemical Composition of Plant Leaf and their Relation with Population Density of Certain Piercing- Sucking Insect Pests

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ABSTRACT

Both leaf morphology and chemistry play a potential role in predicating with the population density of organisms. The current study was conducted, at the experimental farm of Mansoura University, to examine the effect of host plant species and their morphological (trichome density, thickness, and length) and physiological ((i.e., total protein, total carbohydrates, NPK) characteristics on population density of some piercing-sucking insect pests during two successive growing seasons.The number of *Aphis* spp. was positively correlated with the higher ratios of total protein, total carbohydrates, and N of squash leaves. While the numbers of *Bemisia* spp. and *N. viridula,* were positively correlated with the higher ratios of P and K of eggplant leaves. In contrast, *Empoasca* spp. exhibited higher populations on common bean leaves that were poor in all chemical compounds. Host plant species significantly affected the population density of all insects investigated. Squash harbored the higher numbers of *Aphis* spp., eggplant harbored the higher numbers of *Bemisia* spp.and *N. viridula*, and common bean had the higher number of *Empoasca* spp. The populations of aphids, whiteflies, green bugs, and leaf hoppers varied among leaf morphological types. Whitefly and green bugs exhibited significantly higher density on eggplant with more hairiness and hair length, whereas aphids had significantly higher populations on squash leaves with higher hair thickness. Common beans, with lower magnitude of density, thickness and length had the higher populations of leafhoppers. Based on leaf features and their chemical composition, common bean seems to be more resistant against infestation with aphids and whiteflies, squash against green bugs, and eggplant against leafhoppers.

Keywords: Aphids, whitefly, green bug, leafhopper, hairs, trichomes.

INTRODUCTION

Most hemipterans are phytophagous and causevarious crop economic losses. Most notable of the hemipterans are aphids, plant bugs, whiteflies, psyllids and whiteflies. Initially, these insects have classified as minor or secondary pests, but become primary pests because the current changes in agricultural practices, such as the increased use of transgenic and classically plant varieties that resistant to primary pests, and the decrease in chemical insecticide applications (Chougule and Bonning, 2012). Piercing-sucking pests are attacking a wide variety of agricultural crops and causing considerable loss, either directly by sucking plant juice or indirectly as vector transmitting plant diseases (Carter, 1990).

Potassium is a fundamental element for the growth of plants and participates in most of physiological processes (Yoshida, 1981). The mechanism of stomatal opening and closing depends on K (Penny and Bowling, 1974). Potassium improves the translocation the photosynthesis and mobilizes the stored materials (Mengle, 1980). The main function of K is activation of various enzymes (Evans and Sorger, 1966). Deficiency of K has been proved to accumulate soluble carbohydrates, amino acids and reducing sugars. It impairs the synthesis of starch as well as glycogen and blocks the respiratory substances and decreases the rate of oxidative phosphorylation and photophosphorylation (Baskaran *et al*., 1982). Among several environmental consequences, long-term nitrogen (N) loading reduces plant species richness, increases standing crop biomass, and shifts plant composition to a low dominant species (e.g., Aerts and Berendse 1988;Huenneke *et al.,* 1990; Pysek and Leps, 1991; Inouye and Tilman, 1995). In some cases, characteristics of insect communities respond similarly to variations in plant species richness, plant composition, plant productivity and plant tissue quality along N gradients. For example, high N input should increase insect population because this increase as plant species richness decreases, as plant productivity increases, and as plant composition converts to a few dominant plant species with high tissue quality (e.g., Vince *et al*., 1981; Strauss 1987; Sedlacek *et al*., 1988; Andow 1991; Siemann 1998). Thus, the aggregation of insect species or/and natural enemy species in a plant host could be an indication or a reason of host preference by such species due to plant's physiological and behavioural attributes. For example, several factors can affect population density of whitefly in host plants such as Nand Klevels, weather, vigour and age of plant, type and density of trichomes, and natural enemies (Dent, 1995; Leite, 2000).

The surface of plants shapes a framework in which many insects live, feed and reproduce. Leaf surface possessed hairs might impede insects from walking, whereas a smooth surface might make the insects lose their control and fall off the plant. Insects are forced to overcome the challenges posed by various plant surfaces, either behaviourally or morphologically adaptations, to live and reproduce (Southwood, 1986).In response to herbivores, host plants may show morphological (e.g., trichomes, waxes and spines) or chemical defenses (e.g., toxins and digestibility-reducers)(Panda and Khush, 1995). Plant leaf features may impact the preference and performance of herbivores (Gianoli and Hannunen, 2000) and consequently affect the herbivore aggregations (Peeters, 2002). For example, thickness and length of leaf vein affect the oviposition behaviour of the cotton leafhopper (Sharma and Singh, 2002). The epicuticular waxes on plant surfaces can mechanically function against some herbivores by reducing their ability to touch the plants; however, the plant waxes can impede the efficacy of herbivore predators by similar mechanism as well (Eigenbrode, 2004). Plant resistance against herbivores therefore is not necessarily compatible with predation or parasitism because resistance traits may, at the same time, inversely impact both predators and preys, either directly or indirectly (Cortesero *et al.*, 2000; Kennedy, 2003). Hair-like physical structures on plant surface (Plant's trichomes) may help plants to defend themselves against herbivores (Moles and Westoby, 2000; Kennedy, 2003) by impeding insect walking, feeding, and oviposition (Levin, 1973). For example, leaf hairiness delayed induced resistance in *Salix borealis (*Fr.*)* by disturbing the walking activity and feeding behavior of the leaf beetle, *Melasoma lapponica* L. (Zverevа*et al.*, 1998). Although the population dynamics of the current piercing-sucking insects are previously studied on the same or other host plants, continuous monitors for the population at various times and habitats, either on the short- or long-term, would lead to an increase in the successful pest management under various situations and the generation of knowledge of their biology and ecology is necessity to foresee with the pest status.

MATERIALS AND METHODS

The present experiment was carried out at the farm of the Faculty of Agriculture, Mansoura University, during two successive growing seasons 2015 and 2016. Early summer plantation during both seasons on the three vegetables crops, eggplant *Solanum melongena* L., squash *Cucurbita pepo* L. and common bean *Phaseolus vulgaris* L. were applied.

1. Population density of the main piercing-sucking insect pests:

The population density of the main piercing-sucking insects; aphids, *Aphis* spp.; the whitefly, *Bemisia* spp.; leafhoppers, *Empoasca discipiens* Paoli; and the green stink bug, *Nezara viridula* L. was investigated. For each crop, an area of half feddan was splitted into four equal plots during each growing season. The eggplant (variety Black Beauty) was planted at mid-February in the early summer plantation of both season, whereas the squash (variety Hybrid Nancy) and common bean (variety Giza 4) were sown at February 10th during the two seasons of the study. All replicates were received the normal agricultural practices without any chemical applications throughout the whole season.

To estimate the population of aphids (nymphs and adults), leafhoppers (nymphs and adults), the whitefly (immature stages and adult stage) and green stink bug (nymphs and adults), in addition to their associated predators, weekly leaf samples of 20 leaves which representing the upper, middle and lower levels of the plant were chosen at random from each plot. These samples were transferred into paper bags to the laboratory for inspection .The number of insects were recorded by aid of lens 4x and a binocular microscope.

2-Chemical analysis of host plant leaves and its effect on the population density of the main piercing-sucking insect:

To study the relation between the population density of the main piercing-sucking insect pests examined and the chemical constituents of each of the three vegetable crops (common bean, squash and eggplant), chemical analysis of each vegetable crop was taken place in Soil Science Laboratory, Agriculture Research Center, Giza, Egypt for determining the macronutrients (NPK), total protein and carbohydrates. A sample (100 leaves) from each vegetable crop was prepared according to the method proposed by Chapman and Pratt (1961). The nitrogen was estimated by micro keldahl according to Jackson (1979), phosphorus was spectrophotometrically estimated using ammonium molybdate /stannous chlorid method of Chapman and Pratt (1961), and potassium was determined by a flame photometry according to page *et al*. (1982). Theprotein content was estimated by conversion of nitrogen percentage to protein (Kang *et al.*, 2012). Protein % was estimated by the equation = N% × conversion factor (6.25).

3- Effect of plant leaf surface morphology on the population density of the main piercing-sucking insects:

To examine the effect of leaf surface morphology (length, thickness, and density of trichomes) on the population density of the main piercing sucking insects, a fresh sample (10 leaves) from each vegetable crops, representing the lower, middle, and upper sides of the plant, was collected and sent to the electronic microscope lab, Faculty of Agriculture, Mansoura University for measuring the leaf surface hairs (abaxial and adaxial) of each plant species.

4-Statistical analysis

The data obtained were analyzed using one-way ANOVA (CoState), and means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The relation between the population of piercing-sucking pests and plant chemistry (i.e., total protein, total carbohydrates, NPK) is presented in Table (1). The number of *Aphis* spp. was positively correlated with the higher ratios of total protein, total carbohydrates, and N during both growing seasons, which presented in squash leaves. While the numbers of *Bemisia* spp. .and *N. viridula,* were positively correlated with the higher ratios of P and K that analyzed in eggplant leaves. In contrast, *Empoasca* spp. exhibited higher populations during both seasons on common bean leaves that were poor in all chemical compounds analyzed. Host plant species significantly affected the population density of all insects investigated. Squash harbored the higher numbers of *Aphis* spp., eggplant harbored the higher numbers of *Bemisia* spp.and *N. viridula*, and common bean had the higher number of *Empoasca* spp. during both seasons of study (Table 1)

Table 1.The average number (± SE) of certain sucking-piercing insect pestsduring 2015 (A) and 2016 (B) growing seasons at Mansoura region and the percentages of chemical components in various host plants.

Plant species	Average no. of *Aphis* spp.		Average no. of *Bemisia* spp.		Average no. of *N. viridula*		Average no. of *Empoasca* spp.		Chemical constituents %				
	A	B	A	B	A	B	A	B	Total protein	Total carb.	N	P	K
Eggplant	35.2±0.13[b]	32.5±0.57[b]	48.2±0.49[a]	72.8±3.30[a]	4.6±0.13[a]	8.3±0.17[a]	31.6±0.47[c]	27.7±0.33[c]	10.5	45.6	2.85	0.05	7.12
Squash	39.3±0.61[a]	36.3±0.94[a]	43.5±0.66[b]	63.0±3.31[b]	2.2±0.05[c]	2.7±0.18[c]	41.9±1.71[b]	40.7±0.28[b]	11.4	47.1	3.72	0.03	5.52
Common bean	29.6±0.73[c]	24.4±0.34[c]	36.4±0.68[c]	60.7±1.25[c]	4.1±0.09[b]	4.5±0.15[b]	71.8±0.17[a]	66.8±0.88[a]	9.2	41.2	2.45	0.02	0.34

Means bearing the same letters are not significantly different (ANOVA, α = 0.05)

The relation between the population of piercing-sucking pests and plant morphology (i.e., hairs) is presented in Table (2). The morphology of leaf hairs is photographed using Electronic microscope as shown in Figures (1 and 2). Common bean leaves had straight and hooked trichomes on their abaxial and adaxial surfaces, but at relatively low length and thickness. Smooth and soft surfaces with stalked-porrect stellate and sessile-porrect stellate trichomes occurred on adaxial and abaxial eggplant leaves with higher length, at twice the length of common bean and at more than one fold of squash. However, it has lower thickness. Squash leaves characterized by straight acicular and straight trichomes on abaxial and adaxial surfaces with higher thickness and middle length than other two plants. The eggplant had the highest density of trichomes followed by squash and common bean (Table 2). The populations of aphid, whitefly, green bugs, and leaf hoppers varied among leaf morohological features. Whitefly and green bugs exhibited significantly higher density on eggplant with more hairiness and hair length, whereas aphids had significantly higher populations on squash leaves with higher hair thickness. Common beans, with lower magnitudeof density, thickness and length had the higher populations of leafhoppers (Table 2).

Table 2. Comparative morphology of leaves of three vegetable plant leaves.

Plant	Trichome type		Leaf surface	Trichome length µm			Trichome thickness µm			Trichome density mm^2
	abaxial	Adaxial		Abaxial	adaxial	Total	abaxial	Adaxial	Mean	
Eggplant	Stalked stellate	sessile stellate	Smooth	438.09	312.93	375.51	15.42	18.26	16.86	15.53
Squash	Straight acicular	Straight	Ragged	246.70	255.16	250.93	30.94	23.70	27.32	14.52
Common bean	Straight	Hooked	Ragged	89.95	293.15	191.55	9.86	24.59	17.23	9.38

DISCUSSION

The numbers of *Bemisia* spp. And *N. viridula,* were positively correlated with the higher ratios of P and K that analyzed in eggplant leaves. The lowest density of *Bemisia* spp. on sweet pepper can be due to the highest K levels that can minimize the magnitude of cumulated amino acids, which in turn can decline the piercing-sucking insect densities (Leite *et al*., 2011), and thus they suggested that eggplant was the most suitable host for whitefly, as reported in our study. These results, regarding chemical analysis, are inconsistent with our results. The only possible explanation could be because another determined element, that not measured in both studies, responsible for increasing or decreasing insect population, or because the morphology of eggplant leaves, which were more in length and hairs. The higher length and density of leaf hairs could play an important substrate for protecting immature stages of these insects from parasitoid attacks that could be impeded the parasitoid movement). Hairy-leaf cotton, *Gossypium hirsutum* L., cultivars have been colonized with higher *Bemisia* aggregations than those of smooth leaf (Butler and Henneberry, 1984, Butler *et al*., 1991, Flint and Parks, 1990). The lower density of *Bemisia* spp. on sweet pepper may be because the absence of trichomes that their presence could reduce *Bemisia* spp. predation and parasitism (Leite *et al*., 2011). For example, the walking speed of a whitefly parasitoid female, *Encarsia Formosa* Gahan*,* was three-times higher on hairless than on hairy cucumber varieties (Boethel and Eikenbary, 1986).

The number of *Aphis* spp. was higher in squash plants because the higher ratios of total protein, total carbohydrates, and N, and the lower ratio of K and P. According to Jansson and Ekbom (2002), the high level of K can minimize the magnitude of cumulated amino acids that in turn can reduce the population of piercing-sucking insects. Similarity, potassium enters in the synthesis of RNA polymerase and reduces free amino acid levels in the juice of the plant, and thus reduces the populations of *B. brassicae* as well (Marschner, 1995). Although the squash leaves had straight acicular hairs with higher thickness and relatively high trichome density, aphid population was the highest on squash that contained higher ratios of protein and carbohydrates, which both nutrients are more significantly required for aphid development, survival, and reproductive performance. Regardless, the morphology of plant leaf was smooth or ragged, hooked or sharpened, thickened or thinned; plant chemistry seems to be more desired at least by piercing-sucking insects. Furthermore, aphid alates may prefer such leaf surface to i) lay their progeny as a means for protect them from attacking of natural enemies that more likely to impede their activity in aphid searching, and ii) because their richness in nutritional resources. Further, the slow movement of apterous aphid may diminish the impact of such leaf surface. For example, Leite *et al*. (2001) found that higher tomato trichome density can preclude natural enemies from colonized this plant, resulting in the population increase of *M. persicae*; Bayoumy *et al*. (2014) found that the structure of cucumber and castor bean leave surface seems more likely mechanically impeded the walking of *Stethorus gilvifrons*, increased the reactive distance to prey, or both; and Walters (1974) found that the hooked trichomes on *Phaseolus lunatus* L. and *P. vulgaris* L. leaves jagged and penetrated the integument of abdominal segments of three *Stethorus* species larvae, causing significant mortality, and caused injury to adults as well.

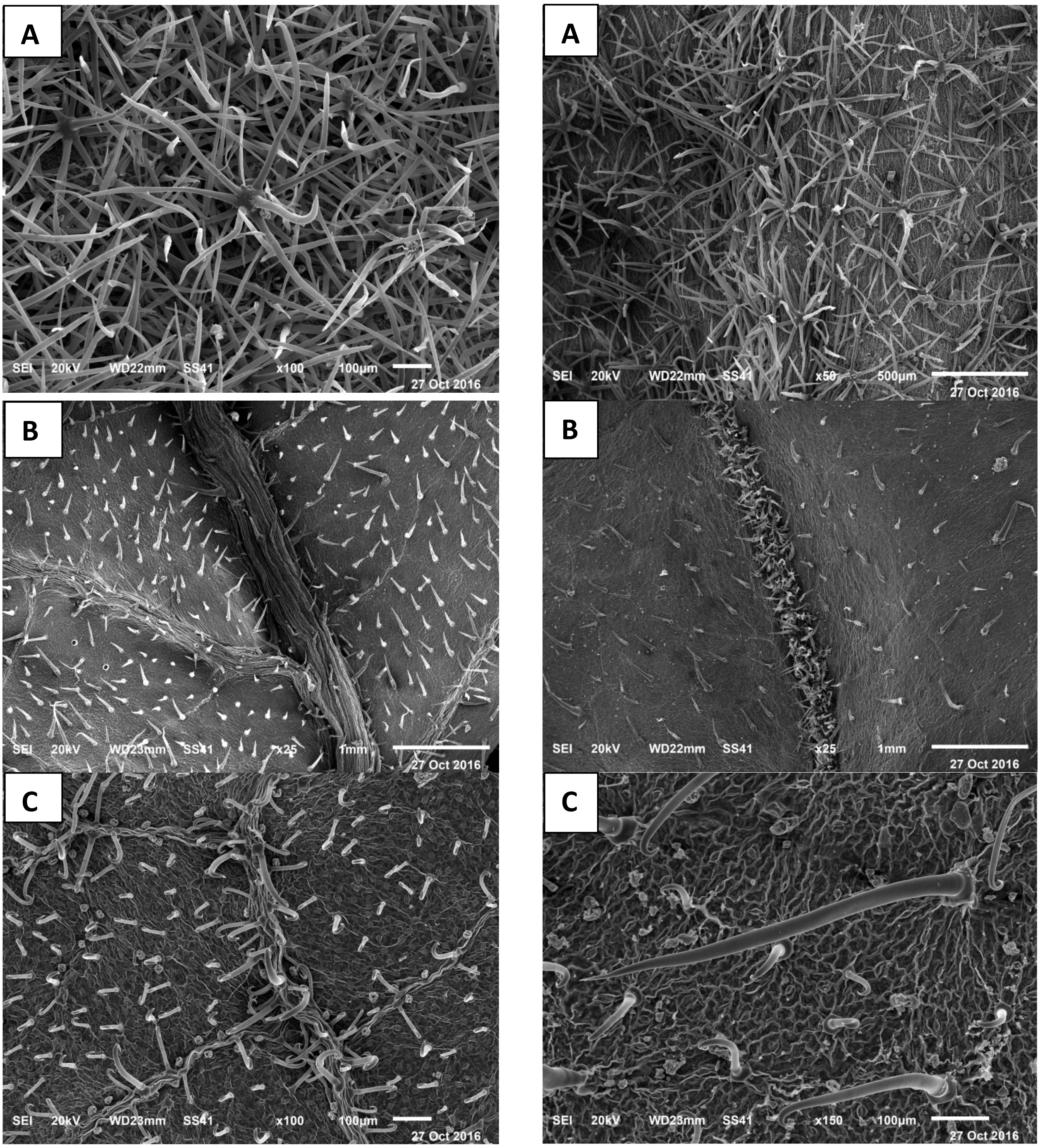

Figure1. Trichome density of eggplant (A), squash (B), and common bean (C) leaves on abaxial, i.e. lower (left) and adaxial, i.e. upper (right) surfaces.

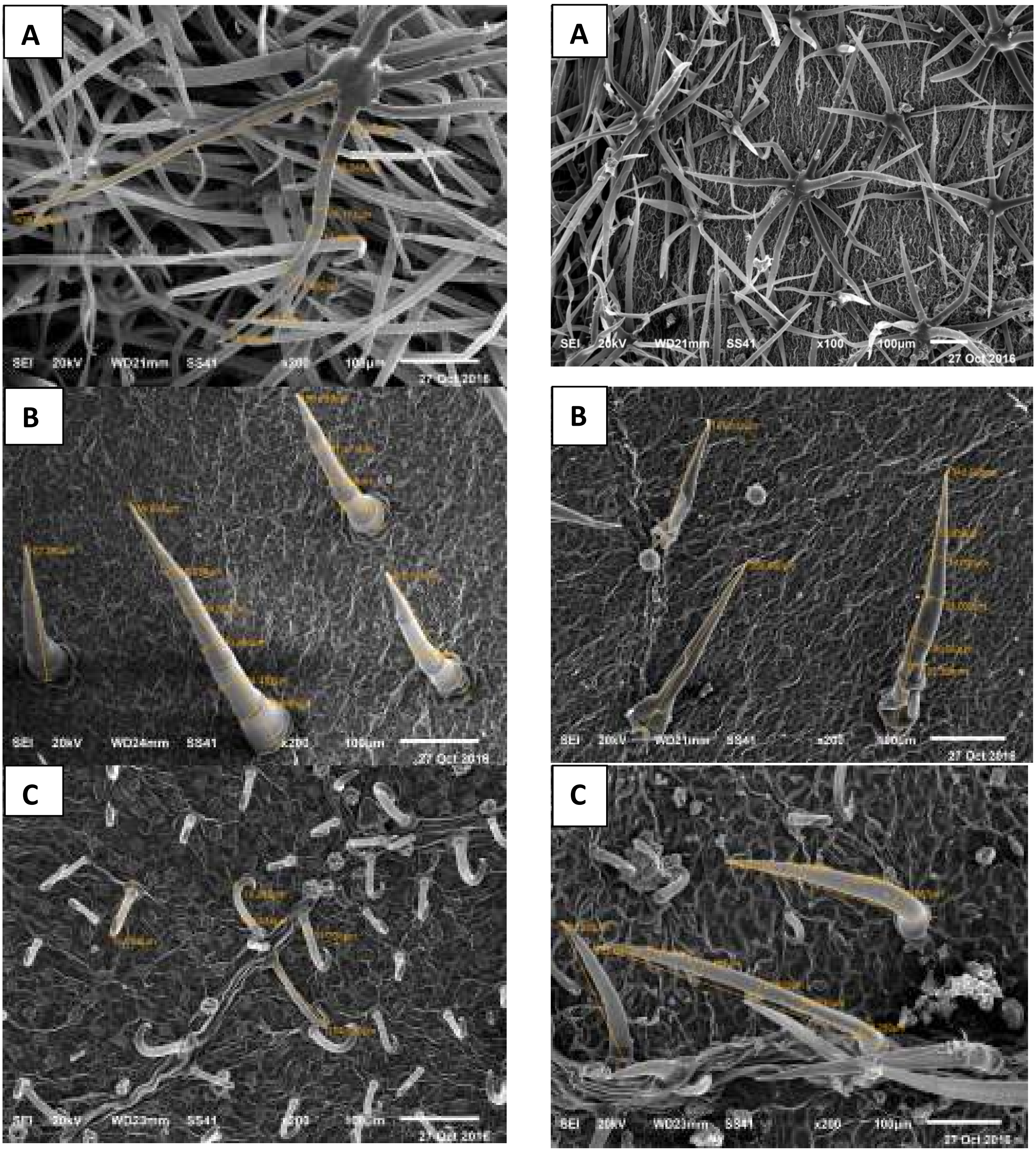

Figure2.Trichome thickness and length of eggplant (A), squash (B), and common bean (C) leaves on abaxial, i.e. lower (left) and adaxial, i.e. upper (right) surfaces.

Although the common bean leaves generally had the lower magnitudes of leaf structures, the leaf hoppers had higher populations on common bean plants despite the poor nutritional resources they have. Jumping legs in leaf hoppers may require ragged surface to easily function, flight, movement from plant to others. The physical structure of plants forms a texture in which insects live and reproduce. A covered surface with trichomes might preclude insects from walking, whereas a smooth surface might make the insects lose their control and fall out the plant. Insects are adapted to face the challenges might pose by the plant surfaces (Southwood, 1986). Lit and Bernardo (1990) found that the two resistant eggplant varieties with longer, more numerous, and more trichomes consistently had fewer leaf hoppers nymphs and eggs. The structures are similar to that described by Metcalf and Chalk (1979). The dense mat of trichomes on leaf surface or thick outer layer of cells may deter the minute early instar nymphs from reaching the feeding sites (Thorsteinson, 1960; Duffey, 1986). Kavousi*et al*. (2009) found that *P. vulgaris* hooked trichomes occasionally trapped and killed adult *Tetranychus urticae* Koch. Another reason for the high populations of leaf hoppers in common bean plants may because the fat effect, which is shortcoming of this study and it is neglected in the analysis. Thus, common bean had lower populations of piercing-sucking insects examined here except the leafhoppers. Lit and Bernardo (1990) found that there was a negative correlation between fat percentages and adult oviposition as well as nymphal feeding preference and suggested that the fats extracted may include more of the resistance factors than nutritional compounds. The fat may contain steroid glycoalkaloids and terpenoids that have been known to occur in many solanaceous species (Tingey, 1984).

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مقارنة المورفولوجي والتركيب الكيماوي لورقة النبات وعلاقتها بكثافة التعداد لبعض الحشرات الثاقبة الماصة للنبات

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يلعب كل من التركيب الكيماوي والمورفولوجي للورقة دور رئيسي في التنبؤ بالكثافات العددية للكائنات الدقيقة. اجريت الدراسة الحالية بالمزرعة البحثية لجامعة المنصورة، لدراسة تأثير الخصائص المورفولوجية (كثافة الشعيرات وسمكها وطولها) والفسيولوجية (كنسبة البروتين الكلية والكربوهيدرات الكلية ونسب النيتروجين والفوسفور والبوتاسيوم) لبعض انواع العوائل النباتيه علي الكثافة العددية لبعض الحشرات الثاقبة الماصة للعصارة النباتية اثناء موسمين زراعة متتاليين من فترة الدراسة. كان تعداد المن مرتبط ارتباطا معنويا ايجابيا بالنسب العالية من البروتين والكربوهيدرات الكلية ونسبة النيتروجين في اوراق الكوسه. بينما كان تعداد الذباب الابيض والبق الاخضر مرتبطا ارتباطا معنويا ايجابيا بالنسب العالية من الفوسفور والبوتاسيوم في اوراق الباذنجان. علي النقيض، سجلت نطاطات الاوراق اعلي تعداد لها علي اوراق الفاصوليا والتي كانت فقيرة في محتواها من العناصر الكيماوية سابقة الذكر. اثرت انواع العوائل النباتية تأثيرا معنويا علي الكثافة العددية لكل انواع الحشرات التي تم تسجيلها، حيث جذبت اوراق الكوسة العدد الأكبر من المن، بينما الباذنجان جذب العدد الأكبر من الذباب الأبيض والبقة الخضراء، في حين جذبت الفاصوليا العدد الأكبر من أنواع النطاطات. حيث اوضحت الدراسة اختلاف تعداد مجاميع المن والذباب الأبيض والبق الأخضر ونطاطات الأوراق طبقا لاختلاف الشكل المورفولوجي للورقة، فالبق الأخضر والذباب الأبيض سجل اعلي كثافة عددية علي الباذنجان الذي يمتلك كثافة شعيرات وطول شعيرات اعلي، بينما سجل المن اعلي تعداد علي الكوسة والتي تمتاز ورقتها بسمك اكبر للشعيرات، في حين ان الفاصوليا التي لديها شعيرات اقل سمكا وطولا وعدداجذبت العدد الأكبر من نطاطات الأوراق. اسنادا الي طبيعة سطح الورقة وتركيبها الكيماوي، تبدو الفاصوليا الأكثر مقاومة للإصابة بالمن والذباب الأبيض، الكوسه الأكثر مقاومة للإصابة بالبق الأخضر، اما والباذنجان الأكثرمقاومة للإصابة بنطاطات الاوراق.