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Allelopathic Indications of Non-polluted and Polluted *Psidium guajava* L. Leaves on some Physiological and Metabolic Aspects of *Vicia faba* L.

Mabroka H. Mostafa and Salama M. El-Darier*
Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

ABSTRACT

The main objective of this study was to determine the impact of allelopathic potentials of non-polluted (reference site) and polluted *Psidium guajava* leaf powder on germination and metabolites accumulation in *Vicia faba*. *Psidium guajava* leaves were collected from Edko (non-polluted) and Amrayya (polluted) regions to represent the two types of leaves respectively. The experimental design was a complete randomized with three replicates. Non-polluted and polluted *P. guajava* dry leaves powder was mixed with sandy loam soil in ratios to get different concentrations (1, 2, 3 and 4%) in addition to soil without powder as control. The dry weight (g seedling^{-1}) and shoot length (cm) of *V. faba* seedlings achieved their reductions at high concentration percentages (3 and 4%). On the other hand, *P. guajava* leaves in the polluted region exhibited more reduction in seedlings root length (cm) compared with the leaves of non-polluted. The decrease in pigment content of leaves as well as carbohydrate and protein content in seedlings shoot and root was remarkable due to polluted rather than non-polluted leaf powder. The concentration of different nutrient elements varied with respect to the element, organ and applied powder. The increased polymorphism and the genomic template stability (GTS) percentages may indicate the adaptation of *V. faba* plant to non-polluted treatments than to the polluted ones. The leaf cells from *V. faba* treated with 4% non-polluted and polluted powders showed marked differences in their metabolic processes that explain the noticeable differences in fine structure of leaf cells.
Keywords: *Psidium guajava*, *Vicia faba*, Growth, Pigments, Nutrients, Protein profile, leaf ultrastructure.

INTRODUCTION

Plants may affect others growing in their vicinity in a stimulatory or inhibitory manner through released biologically active compounds often termed as allelocompounds or allelochemicals. Recently, the phenomenon receiving an increased attention and is considered to be applied in practice for weeds and pest management (Prasanta *et al*., 2008). Plant extract that is not decomposed was thought to contain secondary compounds with allelochemical activity or phytotoxic which cause growth inhibition (An *et al.,* 1993) and alter the available resources in the environment (Wardle *et al*., 1998). Allelochemicals are believed to be a joint action of several secondary metabolites including phenolic compounds (Dalton, 1999), flavonoids (Berhow and Voughn, 1999), juglone (Jose and Gillespie, 1998) and terpenoids (Langenheim, 1994).

It is evident that abiotic stress factors especially industrial pollutants influence growth and secondary metabolite production in higher plants. The influences are well marked as productivities of plants depend on the changed ecosystem (Ramakrishna and Ravishankar, 2011). Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense (Seigler, 1998). Heavy metals contamination in agricultural environments can result from atmospheric fallout, pesticide formulations, contamination by chemical fertilizers, and irrigation with poor quality water (Marcovecchio *et al*., 2007). Heavy metals rank high among the chief contaminants of leafy vegetables, fruit trees and medicinal plants (Ajasa *et al*., 2004).

Psidium guajava L. (guava, Myrtaceae) is a native fruit tree in the tropical rain forest. Its fruits are widely consumed either fresh or processed and it is also used in ethnomedicine for several purposes (Qian and Nihorimbere, 2004).There are two most common varieties of guava: the red (*P. guajava* var. pomifera) and the white (*P. guajava* var. pyrifera) (Haida *et al*., 2011; Kaneria and Chanda, 2011). Guava plants were shown to be effective accumulators of sulfur and fluoride in biomonitoring studies and were sensible to ozone in semi controlled experiments (Rezende and Furlan, 2009; Furlan *et al*., 2010). With this context, *P. guajava* may consider as a bioindicator as they are very sensitive to water and soil pollutants. Such pollutants caused damage of their leaves, impair photosynthetic apparatus and enhance the accumulation of heavy metals and some secondary metabolites as well as alteration of protein pattern in their leaves and fruits (Hemada and El-Darier, 2016).

Therefore, this work aimed to investigate the allelepathic potential of non-polluted and polluted *P. guajava* leaves on germination efficiency, growth and some physiological parameters of *V. faba* (cv. Balady 716, Fabaceae) crop plant as a recipient species. The aim was interprets to validate whether the polluted *P. guajava* leaves elicit harmful effect on the associated broad bean cultivation or not through allelopathic applications.

* *Corresponding authore-mail: salama_eldarier@yahoo.com*

MATERIALS AND METHODS

Sampling and Analysis of Plant Materials

Six *P. guajava* trees were selected during summer 2017 at Edko and Amrayya regions to represent non-polluted and polluted types of trees respectively. The trees are homogenous and more or less at the same age and size. Ten samples from healthy leaves were collected from each tree of the two study sites and thoroughly mixed to obtain a site composite sample. The samples were washed thoroughly with running tap water then distilled water to remove dust particles from leaf surfaces. All samples were dried in an oven at 60°C till constant weight then powdered in an electrical mill.

Pot experiment was carried out to assess the effect of different levels of *P. guajava* leaves crude powder mixed with sandy loam soil (% w/w) on some growth parameters [seedling shoot and root dry weights (g seedling^{-1}) and lengths (cm)] as well as some metabolic and mineral elements constituents (photosynthetic pigments, total carbohydrates and total proteins, and protein profile) in addition to the ultrastructure of *V. faba* plant leaf). To accomplish this aim, five pots of 10 seeds for each tested crude powder: soil levels (0, 1, 2, 3, and 4%) were used. After 21 days, the homogenous seedlings were carefully collected from each treatment; washed with tap water to remove the adhering soil particles and then by distilled water and gently blotted with filter paper.

Determination of photosynthetic pigments of *V. faba* plant leaf (chlorophyll a, chlorophyll b and carotenoids (mg g^{-1} fresh wt. of leaves) were determined spectrophotometrically according to Metzner *et al*. (1965).Total carbohydrates in the dry shoot and root was determined by using a colorimetric method (Herbert *et al*., 1971). Total protein content was determined by the method described by Lowry *et al*. (1951). Concentrations of some mineral elements in the dried shoot and root (at 60°C) were determined using the method of cottenie *et al.* (1982). Sodium dodecylsulfate poly-acrylamide gel electrophoresis (SDS-PAGE) was performed to distinguish and fragment total soluble protein for the different treatments according to the method of Laemmli (1970). Genomic template stability (GTS %) was calculated according to the following equation: GTS = (1-a/n) x100, Where "a" is the average number of polymorphic bands detected in each treated sample and "n" is the number of total bands. Percentage of polymorphism observed in SDS-PAGE profiles was calculated and included disappearance of normal band, appearance of a new band with control profile. Template stability (GTS, a qualitative measure of genotoxic effects) was according to Liu *et al*. (2007). Samples of *V. faba* leaves anatomy were prepared and studied by transmission electron microscope according to Glauret and Glauret (1955) and Mollenhauer (1959).

Statistical analysis of data

All data were subjected where suitable to one or Two-way analysis of variance (ANOVA) according to COSTAT 2.00 statistical analysis software supplied by CoHort Software Company (Zar, 1984). Pair-wise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

RESULTS

Generally, table (1) showed a significant reduction in dry weight (g seedling^{-1}) and length (cm) of *V. faba* shoot as affected with non-polluted and polluted leaf powders concentrations compared to control. The two parameters achieved their marked reductions at higher concentration percentages (3 and 4%). Additionally, the two mentioned parameters for root were found to decrease progressively with the increase in polluted powder percentage relative to control. Commonly, the polluted leaves powder exhibited more reduction compared to non-polluted one.

Table (1): Variation in dry weights (g) and length (cm) of shoot and root of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (%w/w) of non-polluted (NP) and polluted (P) *Psidium guajava* leaves.

Treatment (%)	Dry weight (g)				Length (cm)			
	Shoot		Root		Shoot		Root	
	NP	P	NP	P	NP	P	NP	P
Control	0.26±0.0		0.9±0.01		29.93±0.25		16.80±0.10	
1	0.14±0.10	0.15±0.01	0.06±0.10	0.07±0.02	28.0± 2.00	29.0 ± 0.00	12.95± 0.05	14.5± 0.20*
2	0.05±0.02	0.23±0.01*	0.02±0.02	0.07±0.04	24.7 ± 0.40	28.2 ± 0.30*	11.35± 0.10	13.2±0.10*
3	0.12±0.01	0.09±0.020	0.03±0.01	0.05±0.01	25.67±0.35	24.1± 0.87*	10.60 ± 0.0	11.1±0.17*
4	0.11±0.01	0.02±0.00*	0.04±0.03	0.04±0.02	22.5± 0.10	20.0 ± 0.00*	8.00± 0.870	8.80 ± 0.17
Treatment	F=244.96** (<0.001**)		F=7.33**(0.001**)		F=129.83**(<0.001**)		F=657.06**(<0.001**)	
Concentrations	F=11.31**(0.003**)		F=4.72**(0.042**)		F=0.11(0.747)		F= 73.43**(<0.001**)	
Interaction	F=116.89*(<0.001*)		F=1.51(0.237)		F=15.63**(<0.001**)		F= 9.54**(<0.001**)	

Data was expressed by using mean ± SD., *: Statistically significant with NP ,F: value for two way ANOVA test,**: Statistically significant at $p \leq 0.05$

Table (2): Variation in total photosynthetic pigments (mg g^{-1}fresh weight) of *Vicia faba* leaves germinated in soil supplemented with different concentrations (w/w) of non-polluted (NP) and polluted (P) *Psidium guajava* leaves.

Treatment (%)	Photosynthetic pigments (mg g^{-1}fresh weight)							
	Chl a		Chl b		Carot.		Total	
	NP	p	NP	P	NP	P	NP	P
Control	105.90±0.70		48.30±0.10		14.90±0.40		169.10±0.10	
1	96.3±0.20	89.0±0.70*	52.7±0.10	38.7±0.00*	16.1±0.10	22.8±0.10*	165.1±0.10	150.5±0.10*
2	88.2±0.10	74.5±0.30*	41.6±0.10	30.1±0.10*	15.2±0.20	27.1±0.10*	145.0±0.45	131.7±0.20*
3	80.7±0.17	56.0±0.60*	37.0±0.60	27.6±0.30*	15.9±0.10	30.5±0.00*	133.6±0.10	114.1±.10*
4	72.4±0.0	44.8±0.60*	31.3±0.10	22.3±0.10*	16.8±0.30	43.8±0.20*	125.3±0.0	110.9±0.10*
Treatment	F=9006.06**(<0.001**)		F=9453.231*(<0.001*)		F=3615.991*(<0.001*)		F=90791.819*(<0.001*)	
Concentrations	F=6829.95**(<0.001**)		F=11118.519*(<0.001*)		F=20513.434*(<0.001*)		F=36527.755*(<0.001*)	
Interaction	F=855.27**(<0.001**)		F=808.904*(<0.001*)		F=2857.443*(<0.001*)		F=2558.181*(<0.001*)	

Data was expressed by using mean ± SD., *: Statistically significant with NP ,F: value for two way ANOVA test ,*: Statistically significant at p ≤ 0.001
Interaction (Treatment vs Concentrations)

Table (3): Variation in total carbohydrates and total proteins content (mg g^{-1}dry weight) of *Vicia faba* seedlings, shoot and root, germinated in soil supplemented with different concentrations (w/w) of non-polluted (NP) and polluted (P) *Psidium guajava* leaves.

Treatment (%)	Total proteins (mg g^{-1}dry weight)				Total carbohydrates (mg g^{-1}dry weight)			
	Shoot		Root		Shoot		Root	
	NP	P	NP	P	NP	P	NP	P
Control	199.0±0.0		115.57±0.0		227.11±0.0		126.57±0.0	
1	175.1±0.01	175.3±0.01*	91.1±0.02	111.0*±0.02	197.5±0.02	221.4*±0.01	108.5±0.04	132.7*±0.01
2	160.1±0.01	120.6±0.02*	72.8±0.01	103.0*±0.01	191.6±0.01	230.1*±0.02	93.6±0.02	131.0*±0.01
3	90.8±0.01	171.7±0.0*	66.3±0.01	106.1*±0.01	133.5±0.01	196.6*±0.02	87.1±0.01	117.6*±0.01
4	78.0±0.01	151.8±0.01*	45.1±0.01	95.5*±0.02	116.3±0.02	173.1*±0.02	71.7±0.01	101.4*±0.01
Treatment	F=75469359**(<0.001**)		F=12129306**(<0.001**)		F=34666623**(<0.001**)		F=5180791**(<0.001**)	
Concentrations	F=42839784**(<0.001**)		F=41242338**(<0.001**)		F=47450089**(<0.001**)		F=15344280**(<0.001**)	
Interaction	F=43756115**(<0.001**)		F=3906309**(<0.001**)		F=4667248**(<0.001**)		F=1075074**(<0.001**)	

Data was expressed by using mean ± SD; *: Statistically significant with NP ,F: value for two way ANOVA test,**: Statistically significant at p ≤ 0.05
Interaction (Treatment vs Concentrations)

Pigments (chlorophyll a, b and carotenoids) were decreased due to all concentrations compared to control and the decrease was remarkable for polluted rather than non-polluted leaf powder (Table 2). Data also showed a decrease in total carbohydrates and total protein contents in shoot and root of recipient species and the decrease was more noticeable under polluted compared to non-polluted leaf powder (Table 3).

Variation in the percentages of some nutrient elements in shoot and root of *V. faba* seedlings was illustrated in table (4). Notably, in shoot the two types of leaves significantly increased the content of Na, P, Fe and Ni while the content of Zn and Cu decreased. The concentration of Ca was increased and decreased as affected by the addition of non-polluted and polluted leaf powder respectively. With respect to the root, data showed that the concentration of Na and Cu was increased upon applying the two types of leaf powders while that of Ca was decreased. Regarding Mg and Fe contents, an increase and decrease was exhibited as affected by non-polluted and polluted powders respectively.

Protein in *V. faba* seedlings treated with non-polluted (NP) and polluted (P) *P. guajava* leaves was represented in tables (5 and 6) as well as figure (1). The total number of bands was 30 bands (198 to 28 KDa) with four common bands at 169, 165, 129 and 64 KDa. The specific bands was 3 in 2% NP (181, 145, and 53 KDa), while 4% non-polluted, 2% polluted and 4% polluted treatment were specified with one band for each treatment at 198, 81and 164 KDa, respectively. The maximum percentage of polymorphism was 45% at 2% NP; however the minimum was 29 % at 4% P. The new appearance bands in the protein patterns was of *V. faba* treated with 2 and 4% non-polluted treatments and were 11 and 9 KDa, respectively, while 7 and 6 new bands in 2 and 4% polluted samples, respectively. The disappeared bands at 2 and 4% non-polluted were 5 and 4 bands, respectively and 6 disappeared bands at both 2 and 4% polluted treatments. The genomic template stability (GTS, %) exhibits a considerable variation relative to the control. The 2% of non-polluted treatment attained the maximum percentage of GTS (53.3%). On the other hand, the minimum percentage achieved with 4% of polluted treatment was 40% **.**

Table (4): Variation in the contents (%) of some nutrient elements of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (%w/w) of non-polluted (NP) and polluted (P) *Psidium guajava* leaves.

Treatment (%)	Cu	Zn	Ni	Fe	P	Mg	Ca	Na
Concentration (%) Shoot								
Control	$5.97^{a}\pm0.35$	$3.90^{a}\pm0.17$	$0.30^{a}\pm0.02$	0.20 ± 0.03	$9.5^{c}\pm0.10$	$1.60^{p}\pm0.10$	$3.90^{b}\pm0.10$	$0.10^{c}\pm0.01$
NP	$1.20^{b}\pm0.17$	$0.70^{b}\pm0.10$	$0.70^{b}\pm0.20$	0.50 ± 0.30	$12.5^{b}\pm0.20$	$2.30^{a}\pm0.20$	$4.60^{a}\pm0.10$	$2.30^{a}\pm0.10$
P	$0.80^{b}\pm0.10$	$0.70^{b}\pm0.20$	$0.60^{ab}\pm0.10$	0.50 ± 0.20	$14.4^{a}\pm0.20$	$1.60^{p}\pm0.10$	$1.80^{c}\pm0.10$	$0.60^{b}\pm0.20$
F	455.286^{*}	384.0^{*}	7.753^{*}	2.063	610.333^{*}	24.500^{*}	637.000^{*}	238.922^{*}
p	$<0.001^{*}$	$<0.001^{*}$	0.022^{*}	0.208	$<0.001^{*}$	0.001^{*}	$<0.001^{*}$	$<0.001^{*}$
Concentration (%) Root								
Control	$1.10^{c}\pm0.10$	0.20 ± 0.10	-	$2.90^{b}\pm0.10$	$3.40^{b}\pm0.20$	$2.30^{ab}\pm0.10$	$14.6^{a}\pm0.0$	$1.20^{c}\pm0.20$
NP	$1.70^{b}\pm0.10$	0.20 ± 0.10	–	$4.30^{a}\pm0.20$	$6.90^{a}\pm0.10$	$2.80^{a}\pm0.35$	$12.3^{b}\pm0.0$	$2.20^{b}\pm0.10$
P	$4.20^{a}\pm0.20$	0.40 ± 0.17	-	$2.50^{b}\pm0.30$	$3.40^{b}\pm0.20$	$1.80^{b}\pm0.20$	$10.6^{c}\pm0.1$	$3.67^{a}\pm0.12$
F	405.500^{*}	2.400	–	57.429^{*}	408.333^{*}	13.235^{*}	3627.0^{*}	218.737^{*}
p	$<0.001^{*}$	0.171	–	$<0.001^{*}$	$<0.001^{*}$	0.006^{*}	$<0.001^{*}$	$<0.001^{*}$

F,p: F and p values for ANOVA test, Sig.
bet. grps was done using Post Hoc Test (Turkey)
Means with Common letters are not significant (Means with Different letters are significant)
*: Statistically significant at $p \leq 0.05$.

Table (5): Molecular weights (KDa) of storage proteins in *Vicia faba* seedlings treated with non-polluted (NP) and polluted (P) *Psidium guajava* leaves powder.

Control	Treatment (%)			
	Non-polluted (NP)		Polluted (P)	
	2	4	2	4
-	-	198	-	-
-	193	193	-	-
-	-	189	189	-
-	183	183	183	183
-	181	-	-	-
177	-	-	177	177
-	173	173	173	173
169	169	169	169	169
168	168	168	-	-
-	-	167	167	167
166	166	166	-	-
165	165	165	165	165
-	-	-	-	164
162	-	-	162	-
-	159	159	-	159
150	-	150	-	150
-	145	-	-	-
-	-	141	141	-
-	136	-	-	136
130	-	130	130	-
129	129	129	129	129
-	125	-	113	-
-	-	-	81	-
64	64	64	64	64
-	53	-	-	-
50	-	-	-	50
36	36	36	-	-
34	-	-	-	-
-	28	28	-	-

Table (6): Total number of bands, common band, numbers of specific bands, percentage of polymorphism, appeared (a) and disappeared (b) bands and genomic template stability (GTS%) of storage proteins in *Vicia faba* seedlings treated with non-polluted (NP) and polluted (P) *Psidium guajava* leaves powder.

Character	Treatment (%)				
	Control	Non-polluted		Polluted	
		2%	4%	2%	4%
Total number of bands	13	17	18	14	13
Common band			4		
Specific band	-	3	1	1	1
% of polymorphism	-	42	45	32	29
(a)	-	10	9	7	6
(b)	-	5	4	6	6
GTS%	-	53.3	43.3	43.3	40

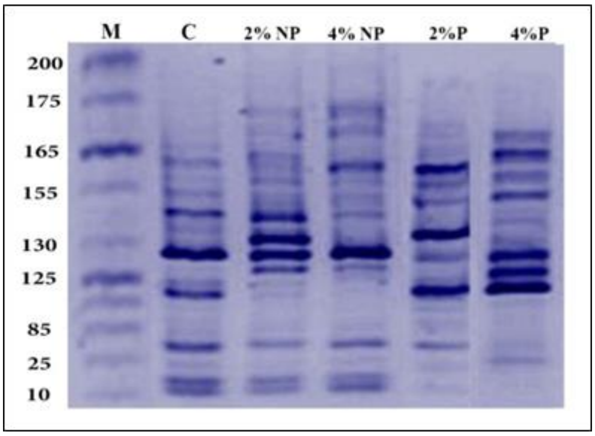

Figure (1): Protein fingerprinting patterns of *Vicia faba* treated with non-polluted and polluted *Psidium guajava* leaf powder.

The electron microscope sections of leaf cells from *V. faba* treated with 4% non-polluted and polluted *P. guajava* leaf powder showed noticeable differences in the fine structure of cells which could be correlated with the marked differences in the metabolic responses of the plant to the imposed stress by different powders. These visible changes (Figure 2**)** when compared to control could be summarized as: 1) the ultrastructural responses of palisade cells are obvious, as 4% polluted leaf treatment injuries appeared in leaves when compare with control. Remarkably, it increased considerably the proportion of chloroplasts showing abnormal shape, swelling or disarrangeement of the lamellae and distorted thylakoids membranes, 2) in the absence of starch, small grana could be seen injured thylakoids (swelling and curling) and high density of stroma (Plate F), 3) the cell wall become thicker with loose fibrillar structures and appearance of a markedly asymmetric cell wall in the 4% polluted leaf treatment, 4) marked dispersed chromatic materials were noticed in the 4% polluted leaf treatment, 5) the nuclei exerted a strong heterogeneous chromatin structure. However dispersed chromatin materials were detected in 4% polluted leaf treatment (Plate C), 6) marked dispersed chromatic materials was observed, thus reflecting the suppression of nucleic acid biosynthesis**.**

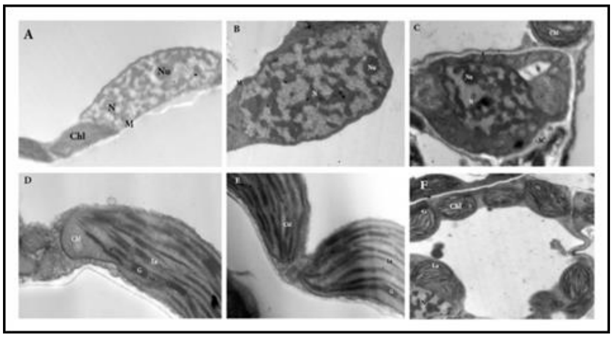

Figure (2): Transmission electron microscopy of the *Vicia faba* leaf : A and D) Control, B and E) 4% unpolluted leaf treatment

DISCUSSION

Allelopathy is a physiological phenomenon with ecological implications (Reigosa *et al.*, 2006) and considered as a sustainable applicable technique in plant ecology. Mixed cropping system (planting two or more crops on the same field) is now more common where cultivation of vegetable and crop species is an old common practice as intercrops in fruit trees orchards (Gliessman, 1985). Industrial activity represents the most significant source of air pollution in the study area at Amrayya, Egypt. An increase in industrial pollution suggests increasing dust deposition onto vegetation and the effect of dust on fruit trees was identified (Braun and Fluckiger, 1987). A wide variety of fruit tree species have been studied in their response to dust which may cause physical injury to tree leaves and bark, reduced fruit setting and a general reduction in growth (Lal and Ambasht, 1984).

Preliminary phytochemical screening of *P. guajava* leaf aqueous extracts showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids (Tambe *et al*., 2014; Hemada and El-Darier 2016). In the same context, extracts contain the phenolic compounds; gallic acid, catechin, epicatechin gallate, syringic acid, *o*-cumaric acid, resveratrol, and Quercetin (Simão, 2017). Commonly, the flavonoids content in leaves showed the tendency of decrease as subjected to industrial air pollution effect (Furlan *et al*., 2010).

The importance of the *V. faba* plant is due to its high nutritive value in both energy and protein contents. Therefore, increasing the crop production is one of the most important targets of agricultural policy in several countries (Mahmoud *et al*., 2004). The current study was an attempt to demonstrate and verify the interfereence of allelochemicals liberated from non-polluted and polluted *P. guajava* leaves with germination efficiency and growth as well as some physiological and biochemical parameters of *V. faba* crop plant. Data exhibited a significant reduction in dry weight and length of *V. faba* shoot and root which was achieved its maximum at higher concentration levels of *P. guajava* leaves from polluted site compared to the not-polluted reference site. In explanation, *P. guajava* leaves subjected to industrial pollution at Amrayya may absorb and accumulate pollutants in their leaves as a phytoremediation strategy. Some studies have already identified *P. guajava* allelepathic effects on other species, for example, the effect of *P. guajava* fruit extracts on cucumber germination (*Cucumis sativus*) (Chapla and Campos, 2010), as well as the effect of root exudates on lettuce (*Lactuca sativa*) germination and root growth, and the root growth of bristly foxtail (*Setaria verticillata*) (Brown *et al.*, 1983). Similarly, *Vicia faba* was sensitive to other plants extracts as the extracts of *Citrullus colocynthis* and this inhibitory effect increased with increasing extract concentration (Salama and Al Rabiah, 2015).

The current work also revealed that leaf pigment content as well as total carbohydrates and total proteins in shoot and root of *Vicia faba* were decreased for all concentrations compared to control and the decrease was remarkable for polluted rather than non-polluted leaf powder. Treatment by *P. guajava* leaf crude powder caused significant increases in the photosynthetic pigments (chlorophyll a, b and carotenoid) of sunflower leaves relative to control (Dawood *et al*., 2012). Salama and Al Rabiah (2015) found the allelopathic effect of *Citrullus colocynthis* shoot extract caused a decrease of carbohydrates and an increase of proteins for *Hordeum vulgare* and *Vicia faba* crop plants. Similar effect was by *P. guajava* which increased significantly the total carbohydrate content in sunflower leaf tissues accompanied with a significant decrease in the total phenolic content relative to control (Dawood *et al*., 2012).

The determination of trace metals is very important because they are involved in biological cycles and indicate high toxicity (Dhiman *et al*., 2011). In the present investigation concentration of Na, P, Fe and Ni increased as affected with the two types *P. guajava* leaves while that of Zn and Cu were decreased. Non-polluted and polluted leaf powder exhibited a different effect on Ca content where increased and decreased values respectively were recorded. The content of Na and Cu in roots was increased upon applying the two types of leaf powders while that of Ca was decreased. Additionally, an increase and decrease attitude for Mg and Fe was exhibited by non-polluted and polluted powders. Nutrients can be stored for later use at a time when the assimilation capacity may be inadequate (Savchuk, 2000). El-Darier (2002) reported that Eucalyptus crude leaf powder significantly increases the accumulation of phosphorus and potassium in leaves of *V. faba* and *Zea mays* while nitrogen was not affected. This nutrients accumulation is one of the most effective mechanisms of phenolic compound actions (Einhellig and Leather, 1988). The present results revealed that allelopathic compound released from polluted *P. guajava* leaves significantly suppressed the accumulation of Zn, Cu and Ca in shoot as well as Mg and Fe in root. Many polyphenols at higher concentrations (4%) have the ability to chelate divalent and trivalent metal ions (Crawley, 1997) which may be coincided with the reduction of growth and development in *V. faba* plant. The same results were obtained by Puri and Khara (1991).

Vegetative storage protein (VSPs) provides the building materials for growth and rapid expansion of vegetative structures after period of dormancy **(**Sözen, 2004). VSPs were first described by Wittenbach (1983) in soybean leaves. Because of their abundance in all vegetative tissues, they have been called vegetative storage proteins. The present study evaluates the VSPs in *V. faba* seedlings treated with non-polluted (NP) and polluted (P) *P. guajava* leaves. Increased percentage of polymorphism indicated the more adaptively of the *V. faba* plant to non-polluted treatments than the polluted ones. Similarly, the increase in GTS indicated that *V. faba* plants were more stable and more adapted to non-polluted treatment than polluted one.

The ultrastructural responses of palisade cells of *V. faba* leaves in the present study increase the proportion of chloroplasts of abnormal shape, swelling or disarrangement of the lamellae and distorted thylakoids associated with high density of stroma. Moreover, the cell wall becomes thick and marked dispersion in chromatin materials led to suppression of nucleic acid biosynthesis. These alterations were prominent in polluted leaves compared with non-polluted. In this context, Lovett (1982**)** observed increased vacuolation and other apparent disruptions in the root tip cells of flax to which an allelochemicals had been applied. As well, phenolic acids retarded hypocotyl growth in *Phaseolus aureus* not through structural damage but rather by interference with mitochondrial respiration (Koch and Wilson, 1977). Leaf cells of *Sorghum bicolor* were less damaged than those of *Zea mays* at low water potentials (Giles *et al*., 1974), and strengthen the hypothesis that maintenance of membrane structure is an important factor in the ability of plants to withstand severe water stress.

CONCLUSION

The pollution impact on secondary metabolism is dependent on pollutant chemistry and on the species studied. Some species activate some biosynthetic pathways, while others are affected in different ways. The present study may indicate that *P. guajava* may consider as a bioindicator as they are very sensitive to different industrial pollutants. Field experiments are needed because under natural conditions the variety of *P. guajava* interactions with the physical environment and other organisms can either intensify or decrease its allelepathic effects. Moreover, the study recommends that *V. faba* was suitable and highly adapted to cultivate as intercrop in non-polluted Guava orchards.

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دلالات الإبعاد التضادي الكيميائي لأوراق الجوافة غير الملوثة والملوثة علي بعض الجوانب الفسيولوجية والأيضية لنبات الفول

مبروكة حميدة مصطفي، وسلامة محمد الضرير
قسم النبات والميكروبيولوجي، كلية العلوم، جامعة الإسكندرية، الإسكندرية، مصر

الملخص العربي

كان الهدف الرئيسي من هذه الدراسة هو تحديد تأثيرات الإبعاد التضادي الكيميائي لمسوق أوراق الجوافة غير الملوثة (الموقع المرجعي) والملوثة على الإنبات وتراكم الأيضات في نبات الفول. وقد تم جمع أوراق الجوافة من منطقة إدكو (غير ملوثة) والعامرية (ملوثة) لتمثيل النوعين من الأوراق على التوالي. وكان التصميم التجريبي العشوائي الكامل مع ثلاثة مكررات هو المستخدم في التجربة. وقد تم خلط مسحوق الأوراق الجافة غير الملوثة والملوثة مع التربة الطميية الرملية للحصول على تركيزات مختلفة (١، ٢، ٣ و ٤٪) بالإضافة إلى التربة بدون مسحوق كمجموعة ضابطة. وقد حقق كل من الوزن الجاف($g\ seedling^{-1}$) وطول (سم) شتلات نبات الفول إنخفاضا في القيم مع نسب التركيز العالية (٣ و ٤٪). ومن ناحية أخرى، فقد أظهرت أوراق الجوافة في المنطقة الملوثة المزيد من الانخفاض في طول جذر الشتلات (سم) مقارنة بالأوراق غير ملوثة.

وكان الانخفاض في المحتوى الصبغي من الأوراق وكذلك محتوى الكربوهيدرات والبروتين في الشتلات في كل من المجموع الخضري والجذور ملحوظا بسبب الأوراق الملوثةمقارنة بمسحوق الأوراق غير الملوثة. أما بخصوص تركيز العناصر الغذائية فقد اختلفت مع إختلاف العنصر والعضو ونوع المسحوق. وقد يشير تعدد الأشكال(polymorphism) الزائد ونسبة استقرار الجينوم (GTS) إلى تكيف نبات الفول مع المعالجات غير الملوثة مقارنة بالملوثة. وقدأظهرت خلايا الفول الورقية والمعالجة بـ ٤٪ من المساحيق غير الملوثة والملوثة اختلافات ملحوظا في عملياتها الأيضية التي تفسر الاختلافات الملحوظة في البنية الدقيقة لخلايا الأوراق.