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Botanical Insecticides and Their Effects on Insect Biochemistry and Immunity

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1. Introduction

Some concerns, especially environmental ones, lead the researchers to find new avenues of insect control in agriculture. Considering negative effects of synthetic pesticides especially on non-target organisms caused a general perception that natural compounds are better products or Generally Regarded As Safe (GRAS) (Scott et al., 2003). So, researches has been concentrated on the plant kingdom for solutions leading to the production of a myriad of secondary compounds that can have toxic, growth reducing, and antifeedant properties against insects (Berenbaum & Zangerl, 1996). The use of plant extracts (botanical insecticides) to protect crops and stored products is as old as crop protection. Indeed, prior to the development and commercial success of synthetic insecticides beginning in the 1940s, botanical insecticides were major weapons in the farmer's arsenal against crop pests (Isman, 2008). Four major types of botanical insecticides are being used for insect control including pyrethrum, rotenone, neem, and essential oils along with three others in limited use (Isman, 2006).

Pyrethrum is an oleoresin extracted from the dried flowers of the pyrethrum daisy, *Tanacetum cinerariaefolium* (Asteraceae) that its active ingredients are three esters of chrysanthemic acid and three esters of pyrethric acid (Isman, 2006). The insecticidal action of the pyrethrins is characterized by a rapid knockdown effect, particularly in flying insects, and hyperactivity and convulsions in most insects. These symptoms are the result of the neurotoxic action of the pyrethrins, which block voltage-gated sodium channels in nerve axons. Azadirachtin is an extraction from Indian neem tree, *Azadirachta indica*has that has two profound effects on insects (Schmutter, 2002). Azadirachtin, apart from its antifeedant effects on insects, inhibited the synthesis and release of ecdysteroids from the prothoracic gland resulting incomplete ecdysis in immature insects and sterility in adult females (Isman, 2006). Rotenone is a type of isoflavonoids extracted from the roots or rhizomes of the tropical legumes like *Derris*, *Lonchocarpus*, and *Tephrosia* (Isman, 2006). Rotenone is a mitochondrial poison by blocking the electron transport chain leading to inhibition of energy production (Hollingworth et al., 1994). Acetogenin extracts from seeds of *Annona squamosa* known as annonin I, or squamocin, and a similar compound, asimicin were isolated from the bark of the American pawpaw tree, *Asimina triloba* (Johnson et al., 2000). Although, there are many plant extracts widely use against insects but here one of them, *Artemisia*, is discussed. The genus Artemisia is a member of a large plant family Asteracea

(Compositae) encompassing more than 300 different species of this diverse genus. Several isolated compounds from this species have shown anti-malarial, antibacterial, anti-inflammatory, plant growth regulatory and cytotoxicity (antitumor) activities (Akhtar and Isman, 2004).

2. Effect of botanical insecticides on digestive enzymes of insects

Digestion is a process in which ingested macromolecules by insects break down to smaller ones to be absorbable via epithelial cells of midgut. Several enzymes based on food materials have critical roles in this process. Any disruption in their activity disables insects to provide their nutrients for biological requirements. Several studies demonstrated the effect of botanical insecticides on feeding parameters of insects by demonstrating food consumption [CR = I/DT], approximate digestibility of consumed food [%AD = 100(I–F)/I], efficiency of converting the ingested food to body substance [%ECI = 100 G/I], efficiency of converting digested food to body substance [%ECD = 100G/(I–F)] and consumption index [CI = I/W] (Shekari et al., 2008). The fact underlying these changes is inhibitory effects of botanical insects on digestive enzymes (Zibaee and Bandani, 2010a).

Starch in plants and glycogen in animals are the storage carbohydrates that amylases are necessary to digest them in herbivorous and carnivorous insects, respectively. *a*-Amylases (EC 3.2.1.1) catalyze the endohydrolysis of long a-1,4-glucan chains such as starch and glycogen (Terra and Ferriera, 2005). Saleem & Shakoori (1987) showed that sublethal concentrations of pyrethroids decreased the *a*-amylase activity in the larval gut of the beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Shekari et al. (2008) demonstrated that a-amylase activity level in elm leaf beetle treated by *A. annua* extract decreased after 24 h and sharply increased after 48 h. Zibaee & Bandani (2010a) showed that *Artemisia annua* extract caused the reduction of a-amylase activity in Eurygaster integriceps Puton (Hemiptera: Scutelleridae), and this reduction increased by higher concentrations of plant extract.

After amylase, glycosidases digest carbohydrate oligomers to monosaccharides (Terra and Ferriera, 2005; Zibaee et al., 2008a; Zibaee et al., 2009a). On the other hands, glycosidases catalyze the hydrolysis of terminal, non-reducing 1, 4-linked alpha-Dglucose residues with releasing of alpha-D-glucose. Treating the adults of *E. integriceps* by different concentrations of *A. annua* extract showed the reduction in the activity of α- and β-glucosidases so that increasing of plant extract concentrations enhanced the enzyme inhibition that emphasais disruption of consumption rates and food conversion efficiencies (Zibaee & Bandani, 2010a). Hemmingi & Lindroth (1999, 2000) determined the effect of phenolic components on gypsy moth (Lepidoptera, Lymantriidae) and forest tent caterpillar (Lepidoptera, Lasiocampidae), founding reduction of the glucosidase activities in both treated larvae.

lipases (EC 3.1.1) are enzymes that preferentially hydrolyze the outer links of fat molecules and have been studied in few insects. Although, there a few studies on insect digestive lipases but the enzyme activity significantly changes due to using botanical insecticides. Senthil Nathan et al. (2006) showed that treating *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), the rice leaffolder, with Btk, NSKE and VNLE (azadirachtin and neem components) sharply decreased the activity level of lipase in the midgut. Zibaee et al. (2008b) found inhibition of lipase activity in the midgut of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) when they add *A. annua* extract to enzyme samples *in vitro*. Zibaee & Bandani (2010a) found similar results when adults of *E. integriceps* fed on food containing *A. annua* extract.

Proteases have a crucial role in food digestion by insects. Different types of proteases are necessary to do this because the amino acid residues vary along the peptide chain (Terra & Ferriera, 2005). There are three subclasses of proteinases involved in digestion classified according to their active site group (and hence by their mechanism): serine, cysteine, and aspartic proteinases. The oligopeptides resulting from proteinase action are attacked from the N-terminal end by aminopeptidases and from the C-terminal end by carboxypeptidases. Studies by Johnson et al. (1990), Senthil-Nathan et al. (2006) and Zibaee and Bandani (2010a) inferred that Botanical insecticides may interfere with the production of certain types of proteases and disable them to digest ingested proteins.

Zibaee et al. (2010) investigated the sole and combined effect of *A. annua* and *Lavandula stoechas* on digestive enzyme activity in *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) (Table 1 and 2). *A. annua* treatment decreased digestive enzyme activities in larvae feed on both mulberry and sycamore in a dos-related manner. Also, treatment of leaves by *L. stoechas* demonstrated a slightly decrease on digestive enzymes except for protease and lipase. However, the effect of L. stoechas extracts on enzyme activities on sycamore was more with regard to mulberry.

Treatment (%)	Esterase		Glutathuion *S*-transferase		Acethylcholinesterase	Alkaline phosphatase	Acid phosphatase
	α-naphtyl	*β*-naphtyl	CDNB	DCNB			
Control	3.76±0.062a	3.34±0.035a	2.82±0.036a	2.84±0.036a	7.56±0.027a	4.92±0.024a	3.93±0.018a
10	4.11±0.021b	3.50±0.012a	2.85±0.022b	2.92±0.022b	7.32±0.020b	4.91±0.020b	3.88±0.046b
15	4.27±0.083b	3.86±0.048ab	3.17±0.031bc	3.27±0.031bc	6.01±0.052c	5.11±0.034c	4.15±0.026c
25	4.75±0.095c	4.22±0.055b	3.49±0.025c	3.45±0.025c	5.31±0.031d	5.46±0.027c	4.35±0.021c

Table 1. Effect of *A. annua* extract on detoxifying enzyme of *E. integriceps* hemolymph after 24 h.

Zibaee & Bandani (2010a) performed analysis of Lineweaver-Burk plots to provide information regarding the mode of action of *A. annua* extract against *E. integriceps* digestive enzymes. In the majority of enzymes, the presence of the plant extract decreased the value of Vmax and increased Km. Since Km has an inverse relationship with the substrate concentration required to saturate the active sites of the enzyme, this indicates decreased enzyme affinity for the substrate (Wilson & Goulding, 1986). In other words, Km is the measurement of the stability of the enzyme-substrate complex and a high Km would indicate weak binding while a low Km would indicate strong binding (Stryer, 1995). The effect of *A. annua* extract on Vmax showed that it interferes with the rate of break down of the enzyme-substrate complex. Thus, the plant extracts inhibit the enzymes by increasing Km and decreasing affinity of the enzyme to substrate. Plant extracts also diminished the Vmax value, which further indicates that they interfered with the rate of breakdown of the enzyme-substrate complex (Morris, 1978). These results showed a mixed inhibition of plant extract on the enzyme activities of the Sunn pest. In this type of inhibition, plant extracts can bind to the enzyme at the same time as the enzyme binds to the substrate, and this binding affects the binding of the substrate and vice versa (Stryer, 1995; Zibaee and Bandani, 2010a). Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect, where the inhibitor binds to a different site on an enzyme. Inhibitor binding to this allosteric site changes the conformation (i.e. tertiary structure e or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced (Morris, 1978; Stryer, 1995; Zibaee and Bandani, 2010a).

Treatment[1]	*a*-amylase		*a*-Glucosidase		*β*-Glucosidase		Protease		Lipase	
	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore
Control	1.87±0.09a	1.75±0.28a	2.05±0.54a	1.90±0.4a	3.88±1.03a	2.67±0.28a	3.80±0.00a	2.36±0.00a	3.43±0.00a	3.34±0.00a
LD_{10}	1.44±0.05b	1.69±0.05a	1.39±0.10b	1.55±0.27b	2.48±0.07c	2.50±0.39b	3.16±0.00ab	1.62±0.00ab	2.79±0.00b	2.61±0.00ab
LD_{30}	1.13±0.00c	1.19±0.04ab	1.24±0.28b	±0.950.09c	1.39±0.14c	1.55±0.21c	1.88±0.00b	0.80±0.00b	2.00±0.00c	2.01±0.00b
LD_{50}	0.84±0.06c	0.99±0.03b	0.52±0.19c	0.14±0.08d	0.28±0.49d	1.25±0.31d	0.76±0.00c	0.50±0.00c	1.47±0.00d	0.56±0.00c

[1]. Concentrations of plant extract are 0.09, 0.22 and 0.42 on mulberry and 0.13, 0.28 and 0.48 on sycamore as . LD_{10}, LD_{30} and LD_{50}.
[2]. Means (SEM±) followed by the same letters above bars indicate no significant difference ($p < 0.05$) according to the Tukey test.

Table 3. Effect of *Artemisia annua* extract on the digestive enzymes profile (*µmol/min/mg* protein) of *Hyphantaria cunea* larvae in the presence of two different host.

Treatment[1]	*a*-amylase		*a*-Glucosidase		*β*-Glucosidase		Protease		Lipase	
	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore	Mulberry	Sycamore
Control	2.08±0.01a	1.97±0.03a	2.20±0.20a	1.49±0.91a	2.89±0.33a	2.61±0.21a	3.64±0.00a	3.51±0.00a	3.25±0.00a	2.77±0.02a
LD_{10}	2.05±0.03a	1.61±0.02b	1.77±0.65b	1.62±0.23	2.42±0.68a	2.46±0.48a	3.65±0.00a	3.43±0.00a	3.18±0.00a	2.49±0.00a
LD_{30}	1.89±0.03b	1.38±0.08b	2.37±0.74a	1.53±0.30a	2.71±0.12a	1.79±0.70a	3.42±0.00a	3.20±0.00a	2.91±0.00a	2.42±0.00a
LD_{50}	1.71±0.02b	1.11±0.05c	2.15±0.75a	1.54±0.34a	2.45±0.23a	1.41±0.23b	3.39±0.00a	3.25±0.00a	2.69±0.00a	2.38±0.00a

[1]. Concentrations of plant extract are 0.02, 0.11 and 0.32 on mulberry and 0.13, 0.38 and 0.79 on sycamore as . LD_{10}, LD_{30} and LD_{50}.
[2]. Means (SEM±) followed by the same letters above bars indicate no significant difference ($p < 0.05$) according to the Tukey test.

Table 4. Effect of *Lavandula stoechas* extract on the digestive enzymes profile (*µmol/min/mg* protein) of *Hyphantaria cunea* larvae in the presence of two different host.

3. Botanical insecticides and detoxifying enzymes

Four types of detoxifying enzymes have been found to react against botanical insecticides including general esterases (EST), glutathione *S*-transferase (GST) and phosphatases.
Esterase (EST) is an important detoxifying enzyme *in vivo* which hydrolyzes the esteric bond in synthetic chemicals. Also, esterase is one of the enzymes showing the strongest reaction to environmental stimulation (Hemingway & Karunatne 1998). The responses of EST to botanical insecticides were significantly due to using different concentrations of extract and long exposure. In the early stage, plant extract stimulated the expression of EST body to increase the detoxification ability (Zibaee and Bandani, 2010b). In the late stage, because of a toxic effect and time EST activity was suppressed.
Glutathione *S*-transferases (GST) are the mainly cytosolic enzymes that catalyze the conjugation of electrophile molecules with reduced glutathione (GSH), potentially toxic substances become more water soluble and generally less toxic (Grant and Matsumura 1989). GSTs play an important role in insecticide resistance and are involved in the metabolism of organophosphorus and organochlorine compounds (Zibaee et al., 2009b). Other xenobiotics such as plant defence allelochemicals against phytophagous insects induce GST activity (Yu, 1982; Vanhaelen *et al.* 2001). By treating *A. annua* extracts on *E. integriceps* adults, Zibaee and Bandani (2010) reported that activity level of GST in 24 h post-treatment increased significantly for both substrates (CDNB, DCNB) of the enzyme. Its (two or one) activity was dose-dependent and increased by exposuring higher concentration of plant extract. Vanhaelen *et al.* (2001) showed that Brassicacea secondary metabolites induced GST activity in *Myzus persicae* and several Lepidopteran species such as *Heliothis virescens* Fabricius, *Trichoplusia ni* Hubner and *Anticarsia gemmatalis* Hubner. The influence of plant

allelochemicals on GST activity is not limited to the herbivores and was observed in several predators, too (Francis *et al.* 2000).

Alkaline phosphatase (ALP, *E.C.3.1.3.1*) and acid phosphatase (ACP, *E.C.3.1.3.2*) are the hydrolytic enzymes, which hydrolyze phosphomonoesters under alkaline or acid conditions, respectively. ALP is primarily found in the intestinal epithelium of animals and its major function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. ALP is involved in the transphosphorylation reaction and the midgut has the highest ALP and ACP activity as compared to other tissues (Sakharov *et al.* 1989). The overall activity of ALP and ACP decreased due to increasing of plant extract concentrations so that there were significant differences among control and three treatments. These findings coincided with other reports of plant extract treatments of insects. For example, Senthil Nathan (2006) showed that treatment of rice plants with *Melia azedarach* Juss (Meliaceae) extracts decreased the activity level of ALP in *Cnaphalcrocis medinalis* (Guenee). These authors reported that feeding *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on *Ricinus communis* L. treated with azadirachtin decreases the amount of this enzyme in the midgut (Senthil Nathan & Kalaivani 2005). Changes in ALP and ACP activities after treatment with *A. annua* extract indicating changes of the physiological balance in the midgut.

4. Botanical insecticides and acethylcholine esterase (AChl)

AChE is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter, acetylcholine, in the nervous system of various organisms (Oehmichen & Besserer 1982; Grundy & Still 1985; Wang *et al.* 2004). Zibaee and Bandani (2010b) demonstrated that *A. annua* extract inhibited the AChE activity in higher doses which coincided with other reports about effect of botanical insecticides on AChE inhibition. The alteration of AChE was observed in the cockroach, *Periplaneta americana* L., at 4 ppm of AZA, (Shafeek *et al.* 2004) and the snail, *Limnaea acuminate* Lamarck, at 40% and 80% concentrations of neem oil (Singh & Singh 2000). It was also observed that 25 g of distilled water extracts of the botanicals *Punica granatum* L., *Thymus vulgaris* L., and *Artemisia absinthium* L., significantly inhibited the AChE activity of nematodes at 100% concentrations (Korayem *et al.* 1993). Senthil Nathan *et al.* (2008) demonstrated that LC50 concentrations of AZA significantly inhibited the activity of AChE compared with control.

5. Botanical insecticides and insect immunity

5.1 Introduction

Similar to vertebrates, insects have a capable immunity against microbial infections exposing in their environment. This immunity based on involved components known as cellular and humeral defenses (Beckage, 2008). Cellular immunity consists phagocytosis of aggressive microorganisms by hemocytes, nodule formation and encapsulation. Humoral responses comprises factors related to the recognition of invading microorganisms, melanization and coagulation as well as killing factors such as antimicrobial peptides (AMPs), reactive oxygen species and reactive nitrogen intermediates, including nitric oxide, prostaglandins and eicosanoids (Boman, 1998; Stanley, 2006; Beckage, 2008).

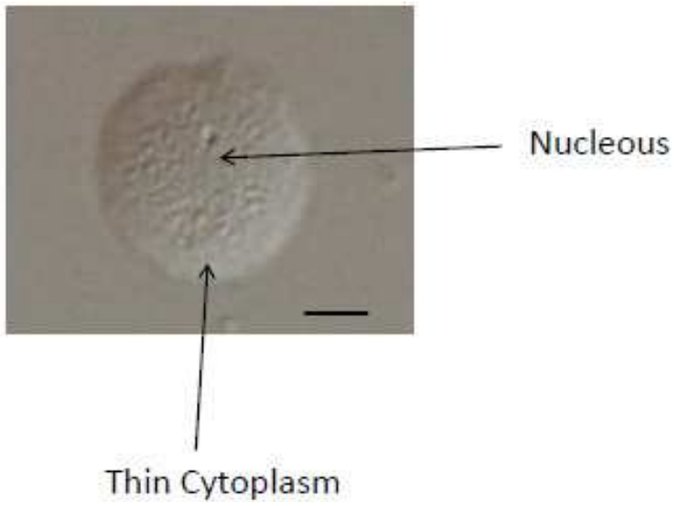
Mentioned immune reactions are initiated by pattern recognition molecules allowing insects to distinguish self-components from nonself-ones. Studies have been identified specific

pattern recognition receptors responding to components in microorganisms such as peptidoglycans and lipopolysaccharides that are main compounds in the cell walls of bacteria and fungi (Theopold et al., 1999; Dziarski, 2004). Peptidoglycan recognition proteins (PGRPs) have been identified in several insect species as activating cascade of melanization on invasive microorganisms (Rolff & Reynolds, 2010). There are specific PGRPs for gram-positive, gram-negative and fungi in the hemolymph of insects. Two signaling pathways namely *Toll* and *Imd* have been activated after recognition of gram-positive microorganisms and fungi as well as gram-negative ones, respectively (Rolff & Reynolds, 2010). These signaling pathways lead to activation of cellular immunity and antimicrobial peptides via final Dif and Relish molecules in nucleus of hemocytes (Tzou et al., 2002; Leihl et al., 2006).
Different environmental factors can definitely affect immune reactions of insects that elucidation of these factors is a significant part to clarify various aspects of these mechanisms. Temperature, different ions and insecticides are some of the most important affecting factors (Zibaee et al., 2009c). In agriculture, combined tactics (as Integrated Pest management) are used to obtain efficient control of insects by considering the lowest disruption in environment. Several studies have been conducted to find combined effect of insecticides, highlighted by botanical materials, and microbial agents on insects. Results revealed that botanical compounds decrease immune ability of insects against microbial agents that describes in forward sections.

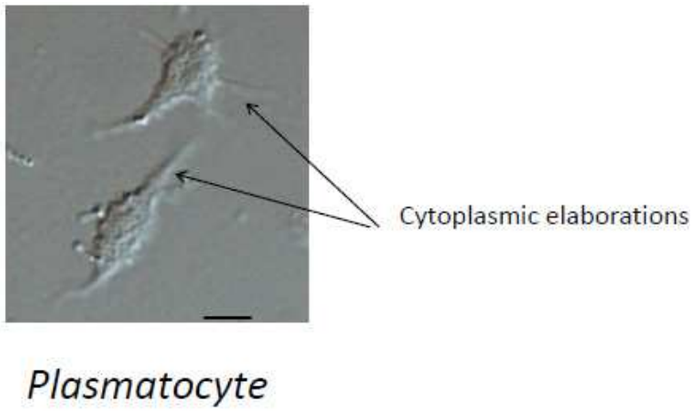
5.2 Effect of botanical insecticides on morphology, number of hemocytes and Phagocytosis

In insect immunity, circulating hemocytes have crucial roles in both cellular mechanisms and producing antimicrobial components. Five basic types of hemocytes have been identified as prohemocytes, plasmatocytes, granulocytes, adipohemocytes and oenocytoids (Lavine & Strand, 2002). Prohemocytes as the smallest one are the basic hemocytes that developed to plasmatocytes and granulocytes when an infectious challenge appeared in the hemolyph. They recognized as large central nucleus and narrow cytoplasm (Lavine & Strand, 2002; Zibaee & Bandani, 2010a) (Figure 2a). Plasmatocytes and granulocytes are the important hemocytes in immune responses to pathogens via phagocytosis (Granulocytes and relatively Plasmatocytes), nodule formation and encapsulation (Strand, 2008). They discriminate each other by spindle shape of plasmatocytes and rounded granulus granulocytes (Figure 2b and c). Adipohemocytes contain lipid droplets so some literature consider them as fat bodies instead of hemocyte (Figure 2d) (Beckage, 2008). Oenocytoids have two specific shape based on intact and immune challenged insects. In normal situation, oenocytoids are spherical cells with peripheral nucleus and crystalline inclusions without any granules (Figure 2e). When an immune challenge occurred, nucleus is going to be smaller and granules appear showing their crucial roles in phenoloxidase[1] (PO) cascade (Strand, 2008; Beckage, 2008).
Different environmental factors could affect insect hemocytes both morphologically and functionally. For example, elevation of environmental temperature increases numbers of plasmatocytes and granulocytes up to 30-40 °C in addition their nodulation ability (Zibaee et al., 2009). Also, different divalent cations have positive effect on hemocytes to provide a cellular network entrapping pathogens in the hemolymph (Willot et al., 2002; Willot and Tran, 2002; Zibaee et al., 2009c) (Figure 3).

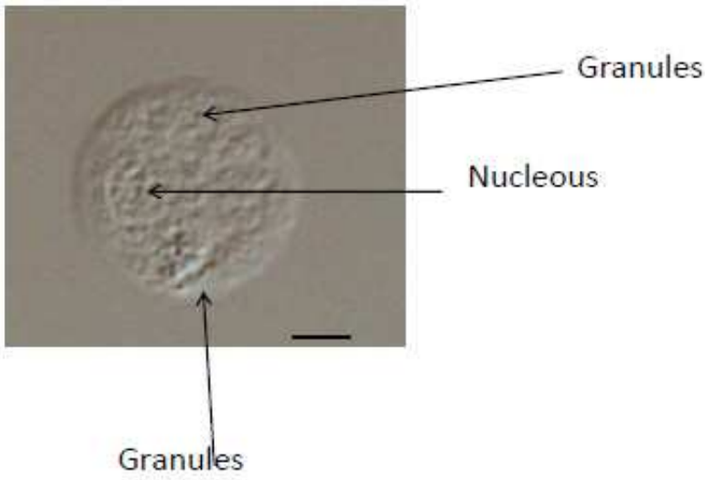
[1] Phenoloxidases have crucial role in immune recognition pathways and melanization of nodules and capsules around an pathogens.


Nucleous
Thin Cytoplasm

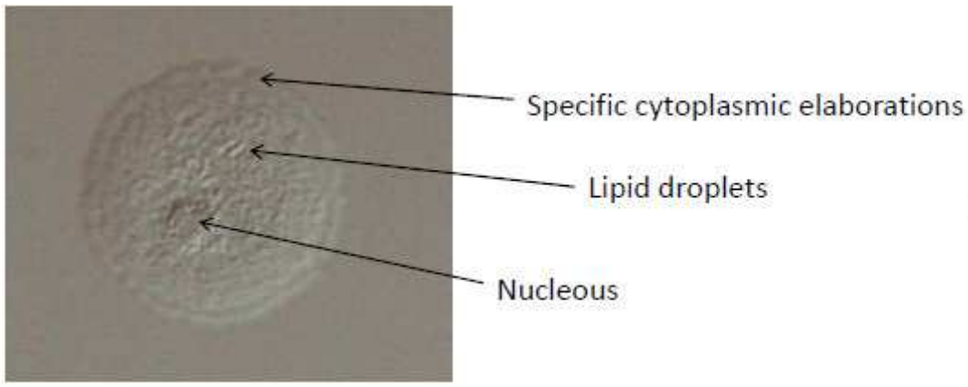
(a)


Cytoplasmic elaborations
Plasmatocyte

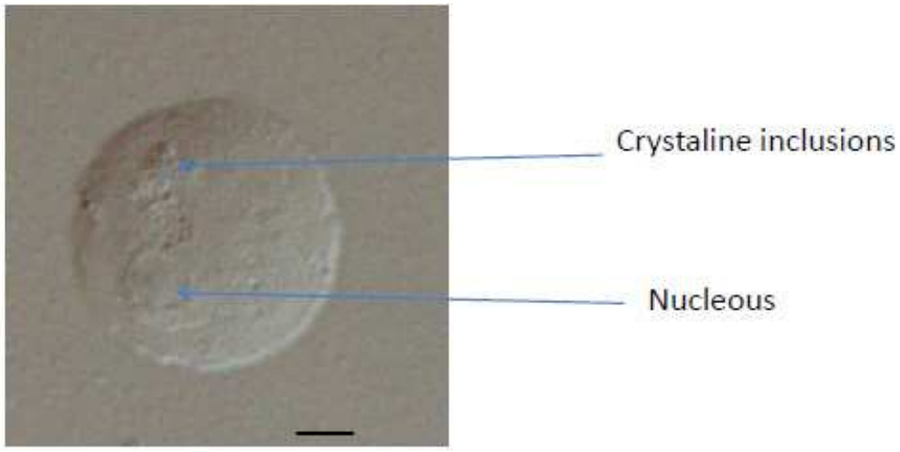
(b)


Granules
Nucleous
Granules

(c)



(d)



(e)

Fig. 2. (a-e) Light microscopy of *E. integriceps* hemocytes. (a) A prohemocyte with a large nucleus (thin arrow) and a thin cytoplasm (b) A plasmatocyte exhibiting a spindle shape and cytoplasmic elaborations. (c) A granulocyte filled with the typical granules in the cytoplasm (arrow) and large nucleus (arrow). (d) An adipohemocyte with lipid droplets spreading in the cytoplasm and specific cytoplasm elaborations (e). An oenocytoid with a round eccentric nucleus and crystalin inclusions. Magnification 40X with the exception of (b) (60X). Bar= 50 µm, with the exception of (b), 33 µm. (Zibaee, A., Bandani, A. R., Talaei-Hassanlouei, R. & Malagoli, D. et al., Unpublished data).

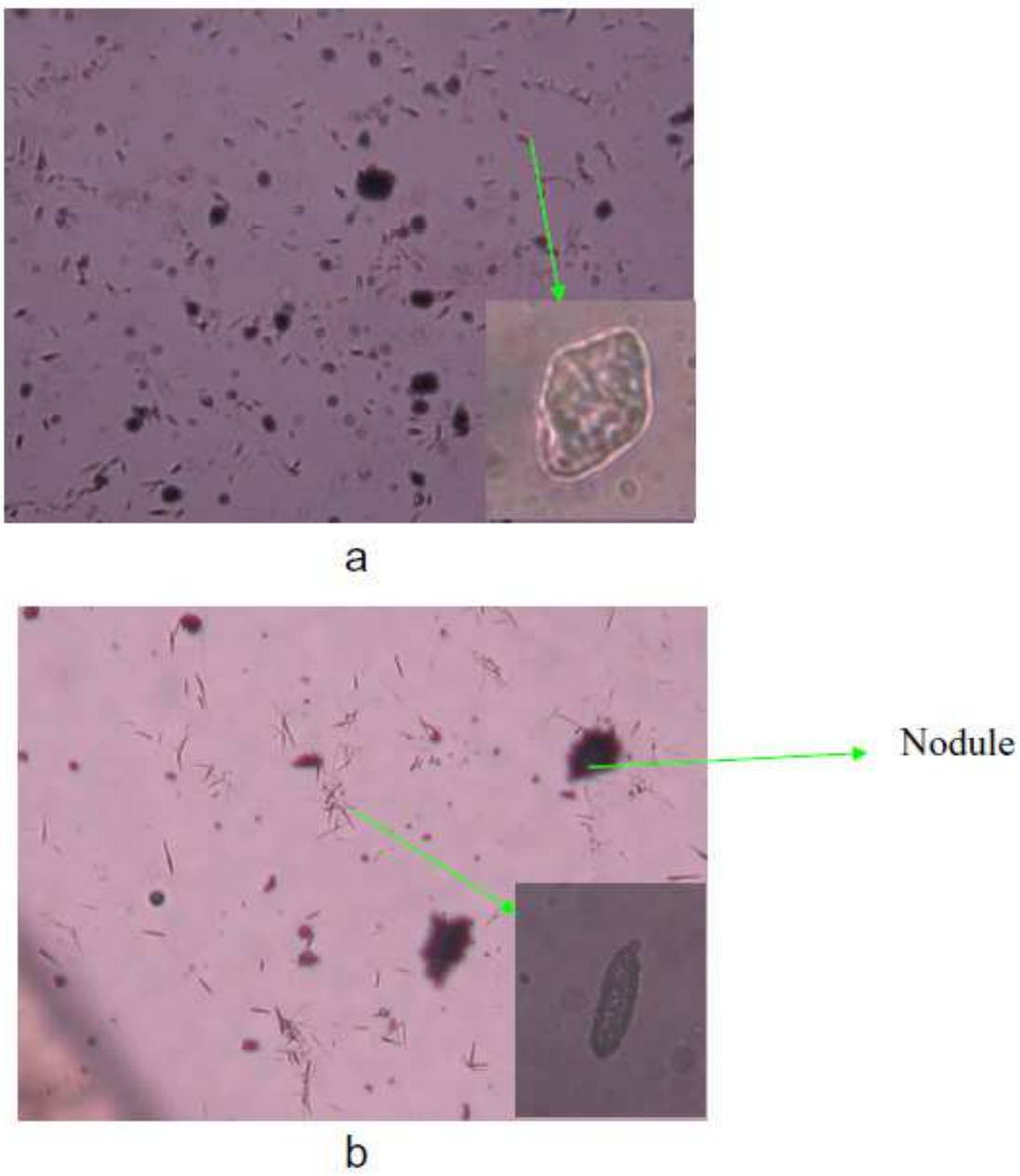


Fig. 3. Phase contrast microscopy of plasmatocytes incubated 12 h by calcium. (a) Control plasmatocytes without incubation by calcium. (b) plasmatocytes incubated by 5 mM concentration of calcium (Zibaee et al., 2009c; Entomological Research; Wiley-Blackwell publishing).

In addition of these positive factors on hemocytes of insects, several other factors, mainly insecticides, have negative effects on number and morphology of them (Figure 4). There are some reports on effects of plant products on the hemocytes such as *Periplaneta americana* L. (Blattodea: Blattidae) (Qadri & Narsaiah, 1978), *Dysdercus koenigii* Fabricius (Hemiptera: Pyrrhoeoridae) (Saxena & Tikku, 1990; Tikku et al., 1992), *Cyrtacanthacris tatarica* L.

(Orthoptera: Acrididae) (Peter & Ananthakrishnan, 1995) and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) (Sharma et al., 2001, 2003, 2008). Studies by scan electron microscopy demonstrated the complete loss of filopods in plasmatocytes and cytoplasmic projections in granular hemocytes of *S. litura* larvae treated with Neem gold (Sharma et al., 2003). Sharma et al. (2008) also find similar results on effect *Artemisia calamus* oil on larvae of *S. litura* as loss of cytoplasmic projections in granular hemocytes. Interestingly, they observed vacuolization in the cytoplasm and degeneration of the organelles, both in plasmatocytes and granular hemocytes (Sharma et al., 2003). So, it was concluded that rapid degeneration of granular hemocytes, initiated by vacuolization and loss of firmness of organelles leading to degranulation and a degenerative transformation within a period of 48 h, subsequently resulting the total collapse of immunity-building mechanism of *S. litura* (Sharma et al., 2008).

Atemisia annua extract altered number of hemocytes and their phagocytic activity in *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Zibaee & Bandani (2010a) reported that treatment of *E. integriceps* with *A. annua* extract affected the total number of hemocytes circulating in the hemolymph indicating that the responses could be due to the toxic effect on the immune cells reducing number of hemocytes attached to fungal spores. Meanwile, an extremely low phagocytic activity was observed in these bioassay experimental groups. Since the attachment of fungal spores to the hemocyte surface is an essential prerequisite to the triggering of phagocytic responses, suggesting that the cellular activity or recognition of spore by hemocyte receptors may be compromised in the hemocytes of insects treated with *A. annua*. Phagocytosis of microbial cells involves interactions between lections on phagocytic cells and sugars on microbial surfaces (Nayar & Knight, 1997). Since *A. annua* extracts suppress phagocytosis (and also nodule formation and PO activity) at different concentrations, this suggests that it may interfere with the ligend-receptor interactions that are likely to occur at the plasma membrane of specific hemocytes because the majority of interactions between cellular and humeral components of the insect immune system are receptor-mediated (Ratcliffe & Rowley, 1987). Therefore, plant extracts at the sub-lethal levels might be enough to interfere with the function of specific receptors, e.g. b-1,3-glucan-specific protein of many insect-species hemocytes, or cause ultrastructural alteration which interfere with normal hemocyte function (Vey et al., 2002).

Garcia et al. (2006) reported significantly higher numbers of *Trypanosoma rangeli* in the hemolymph of *Rodnius prolixus* L. (Hemiptera: Reduviidae) fed on blood containing physalin B at days 2, 4, and 6 post-injection in contrast to that observed in the control. In fact, their data supported the hypothesis that physalin B is an immunomodulator to *T. rangeli* challenge in *R. prolixus*. They concluded four main points for verifying this hypothesis. (i) Mortality of *R. prolixus* in response to common parasite challenge was expressed in a concentration-dependent way in insects treated with concentrations ranging from 0.01 to 1 µg of physalin B/ml of blood meal the idea was supported by Zibaee & Bandani (2010a). (ii) The death rate was significantly enhanced in insects that received concentrations of 0.1 and 1 µg of physalin B and were infected with flagellates. (iii), the hemocyte microaggregation response and nitrite/nitrate concentration, which represent metabolic products of nitric oxide reactions and RNI metabolism against *T. rangeli* infection, was significantly reduced in the hemolymph of insects treated with physalin B (0.1 µg /ml) when compared with infected untreated controls. (iv) The number of parasites in the hemolymph of treated-insects was significantly higher than that observed in insects feeding on blood without physalin B. Based on these results, they proved that physalin B is a

regulator of microaggregation and nitric oxide reactions to parasite challenge in 5th-instar *R. prolixus* larvae (Garcia et al., 2006).

5.3 Effect of botanical insecticides on nodule formation and phenoloxidase activity

PO enzymes in hemolymph that have tyrosinase-like activity can hydroxylate tyrosine (*EC* 1.14.18.1) and also can oxidize *o*-diphenols to quinones (*EC* 1.10.3.1) (Gorman et al., 2007) so called *o*-phenoloxidases. The quinones produced by PO undergo a series of additional enzymatic and non-enzymatic reactions leading to polymerization and melanin synthesis in the final stages of nodulation and encapsulation against invading microorganisms (Zibaee et al., 2011). In fact, insect PO are synthesized as zymogens called pro PO which are activated by proteolytic cleavage at a specific site in response to infection or wounding (Cerenius & Söderhall, 2004). Active PO catalyzes the formation of quinones, which undergoes further reactions to form melanin (Cerenius and Söderhäll, 2004; Gorman et al., 2007). Zibaee & Bandani (2010a) showed the negative effect of *A. annua* extract on nodule formation and phenoloxidase activity of *E. integriceps* (table 2 and 3). Lineweaver-Burk plots analysis of PO activity after treating insects by plant extract revealed an inhibition on enzyme activity by decreasing V_{max} value and increasing K_m one. Since the K_m has an inverse relationship with the substrate concentration required to saturate the active sites of the enzyme, this indicates decreased enzyme affinity for substrate (Zibaee et al., 2011). In other words, K_m is the measurement of the stability of the enzyme-substrate complex and a high K_m would indicate weak binding and a low K_m strong binding. The effect of *A. annua* extract on the V_{max} shows that it interferes with the rate of break-down of the enzyme-substrate complex. Thus, plant extract inhibit the enzymes by increasing the K_m and decreasing affinity of the enzyme to substrate. Plant extract also diminished the V_{max} value which further indicates that they interfere with the rate of breakdown of the enzyme-substrate complex. These results showed a mixed inhibition of plant extract on the enzyme activities of the Sunn pest. In this type of inhibition, plant extract can bind to the enzyme at the same time as the enzyme binds to substrate and this binding affects the binding of the substrate, and vice versa. Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect where the inhibitor binds to a different site on an enzyme. Inhibitor binding to this allosteric site changes the conformation (i.e., tertiary structure e or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced (Zibaee et al., 2011).

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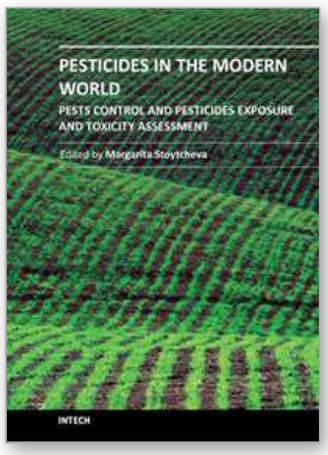

Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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