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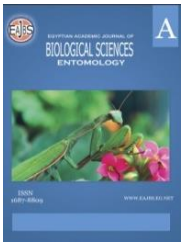

Disturbing Effects of Botanicals on the Haemogram and Immune Parameters of Insects: Recent Progress of The Search for Effective Biopesticides

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ABSTRACT

The excessive and indiscriminate uses of synthetic insecticides usually lead to many problems of human health, environment and economics. Therefore, it is a prerequisite to search for safe alternatives among which plant extracts and products represent effective materials for pest control. The main goal of the present article was searching for new control materials of the insect pests *via* the disruptive effects of these materials on the haemogram and immune parameters. In this article, we discussed the drastic effects of botanicals on the major haemogram parameters including total hemocyte population, differential hemocytes counts, cytopathological deformities of hemocytes, haemolymph (blood) volume, mitotic index and heart activity. It focused, also, on the innate immune reactions (humoral and cellular) in insects and the serious impacts of botanicals on their mechanisms (phagocytosis, encapsulation, nodulation and melanization). As concluded in the current article, botanicals inhibit the immune capability, leading to the insects become susceptible to the action of pathogenic microorganisms and ultimately death. This can be appreciated as a new strategy for the effective control of insect pests. However, some points of future research had been provided. In addition, some field works should be conducted to realize the botanical potential of the haematological and immunological criteria for insect pest control.

Contents:

List of Initials and Abbreviations:

Abscisic acid (**ABA**), adiphohaemocytes (**ADs**), azadirachtin (**Azt**), blood volume (**BV**), coagulocytes (**CGs**), Differential haemocyte count (**DHC**), gibberellic acid (**GA_3**), granulocytes (**GRs**), haemolymph volume (**HV**), 20-hydroxyecdysone (**20E**), indole-3-acetic acid (**IAA**), juvenile hormone (**JH**), Mitotic index (**MI**), oenocytoids (**OEs**), phenoloxidase (**PO**), plant growth regulator (**PGR**), plasmatocytes (**PLs**), prohaemocytes (**PRs**), prophenoloxidase (proPO), scanning electron microscope (**SEM**), spherulocytes (**SPs**), Total haemocyte count (**THC**).

INTRODUCTION

Synthetic insecticides play an important role in the integrated pest management programs and the reduction of insect-borne diseases for more than a half-century and their uses may remain necessary for some years in the future. Nonetheless, the excessive and discriminate uses of many conventional insecticides lead to many drastic problems, such as environmental pollution, hazards to animals and human, destruction of the pollinators and all other non-target insects as well as the natural enemies, like parasites and predators (Miles and Lysandrou, 2002; Jeyasankar and Jesudasan, 2005; Aydin and Gurkan, 2006; Davies *et al*., 2007; Costa *et al*., 2008; Relyea, 2009; Mosallanejad and Smagghe, 2009; Sabry and Abdel-Aziz, 2013; Vendan, 2016). Also, the indiscriminate and excessive use of these insecticides has resulted in outbreaks of secondary pests (Dubey *et al*., 2011). The World Health Organization estimated to have 2,00,000 people are killed by chemical insecticides worldwide (CAPE, 2009) and due to a higher dose and repeated application, every year one million people suffer from pesticide poisoning, cardiopulmonary, neurological and skin disorders, fetal deformities, miscarriages, reduction of the sperm count of applicators (Abhilash and Singh, 2009).

Therefore, many researchers and institutions worldwide are seeking for less persistent and biodegradable alternative materials to minimize these drastic toxicological problems to humans and the environment and to retard the development of resistance in insects (Hussain, 2012; Salama *et al*., 2013; Derbalah *et al.*, 2014). In the last 40 years, the increasing research for extracts and plant-derived materials as pest control agents led to what could be considered the second era of biopesticides (Silva *et al*., 2002; Clemente *et al*., 2003). As reported by several authors (Farag, 2002; Sadek, 2003; Koul, 2004; Niroula and Vaidya, 2004; Viglianco et al., 2006; Strand, 2008; Tavares et al., 2009; Dubey *et al*., 2010; Vogelweith *et al*., 2011; Lampert, 2012; Bokaeian *et al*., 2013; Hamadah *et al*., 2013; Adlin *et al*., 2015; Ben Hamouda *et al*., 2015; Chennaiyan *et al*., 2016a,b), extracts of many plant species and some plant-derived products represent an effective alternative

for pest control; since they exhibit different effects on many insects, as growth regulators, antifeedants, toxicants, repellents, oviposition deterrents, suppressors of reproductive behavior, fecundity and fertility, with low pollution, quick degradation in the environment, less expensive, more effective, less toxic to natural enemies and mammals, safe for human and more selective in action than synthetic pesticides. Thus, the application of materials of the plant origin is considered environmentally and medically safe (Dayan *et al*., 2009; Lokesh *et al*., 2017).

Over the last few decades, the worldwide research on insect circulating haemocytes has received much attention because these cells are responsible for different physiological functions, such as cell development and differentiation; metabolic processes; reproductive potential; endocrine regulation; distribution of nutritive materials and hormones to various sites throughout the insect body; coagulation to prevent loss of blood and wound healing; preservation of insect homeostasis; defense reactions against natural enemies invading the insect body cavity; as well as the detoxification of metabolites and other foreign materials (for some detail, see: Garcia and Rosales, 2002; Ceraul *et al*., 2003; Zhou *et al*., 2004; Ling *et al*., 2005; Ribeiro and Brehelin, 2006; Suhail *et al*., 2007; Strand, 2008; Pandey *et al*., 2010, 2012; Shaurub, 2012; Soares *et al*., 2013; Siddiqui and Al-Khalifa, 2014; Ghoneim *et al*., 2015a,b,c,d, 2017; Chavan *et al*., 2017; Er *et al*., 2017). In addition to these functions, insect hemocytes are very important for clearing apoptotic cells during development (Kurtz, 2002), as well as they are used as an excellent model system for the study of cell communication (Manogem *et al*., 2015). Some information has been available on the effects of botanicals on the haemocyte populations and their ultrastructural composition in several insects (Tikku *et al*., 1992; Vey *et al*., 2002; Sharma *et al*., 2003; 2008).

From the immunological point of view, hemocytes are very vital components of the insect immune system and are biochemically very sensitive having multiple functions, such as phagocytosis, encapsulation and nodulation, as defence mechanisms (Sorrentino *et al*., 2002; Ceraul *et al*., 2003; Ling *et al*., 2005; Figueiredo *et al*., 2006; Gandhe *et al*., 2007; Singh *et al*., 2008; Pandey *et al*., 2010, 2012; Vogelweith *et al*., 2016). In the last few decades, researchers are devoting a great deal of work to haemocytes and their role in insect immunity (Jones, 1959; Gupta, 1979, 1985; Charalambidis *et al*., 1996; Tiwari *et al*., 2002; Ceraul *et al*., 2003; Pandey, 2004; Ling *et al*., 2005; Figueiredo *et al*., 2006; Gandhe *et al*., 2007; Merchant *et al*., 2008; Charroux and Royet, 2009; Pandey et al., 2010; Pandey and Tiwari, 2011; Shaurub, 2012; Ajamhassani *et al*., 2013; Blanco, 2016; Asiri, 2017). The primary goal of this article was the review of disruptive effects of different botanicals on the hematological criteria as well as their histopathological impacts on circulating hemocytes. It secondarily discussed the efficacy of botanicals against the cellular defense reactions in insects. In addition, one of the main objectives in the present article was searching for new control strategies of insect pests basing on the disruptive effects of plant products on haemogram parameters and immune reactions.

2. Total Hemocyte Population as Influenced by Botanicals:

As previously reviewed, circulating hemocytes play a prerequisite role in insect physiology. For example, they are responsible for different functions including general and specific immune response to infection, such as phagocytosis, encapsulation and nodulation (Gregoire, 1957; Wigglesworth, 1973; Gupta, 1985; Lackie, 1988; Millar and Ratcliffe, 1989; Xylander, 1992, 2009; Baishya *et al*., 2015). Within the same insect species, hemocyte population varies over development and environmental adaptability in response to stress (Ratcliffe, 1985; Bergin *et al*., 2005; Brayner *et al*., 2007). Therefore, any stress condition resulting in changes in the total hemocyte population, hemocyte morphology and

functions would ultimately have an adverse influence on the overall physiology and survival of the insect (Sharma et al., 2008; Feitosa *et al*., 2015; Haszcz, 2016; Baishya *et al*., 2016).

'Haemogram' is a term being coined for the haemocyte population picture in an insect at a given time. It is a quantitative (Total haemocyte count, THC) and qualitative (Differential haemocyte count, DHC) expression of the haemolymph and its constituents and inclusions (Jones, 1962; Wheeler, 1963; Jones, 1967a, b; Arnold, 1972). Haemogram parameters include, also, haemolymph (blood) volume, mitotic index and cytological features of hemocytes. In his comprehensive article, Ghoneim (2019) discussed different factors affecting the THC in insects, such as the developmental stage, sex, age, circadian rhythm, reproductive status, temperature, moisture, habitat topography, nutrition, behavioral pattern and the measuring technique. In the following sections, THC fluctuations in different insects have been discussed in relation to the insect responses to many plant products.

2.1. Increasing THC:

Depending on the available literature, there are many reported results of increasing THC in different insects as a response to botanical treatments. For example, THC in the haemolymph of the Egyptian cotton leafworm *Spodoptera littoralis* increased after treatment with azadirachtin (Azt) and Margosan-O (neem formulation) (Rizk *et al*., 2001) as well as with some compounds derived from urea waste and rice straw (Hassan *et al*., 2013). THC increased in the black cutworm *Agrotis ipsilon* larvae after treatment with acetone extract of *Melia azedarach* (El-Shiekh, 2002; Shaurub and Sabbour, 2017). THC increased in the seven-spot ladybird *Coccinella septempunctata* after treatment with Azt (Suhail *et al*., 2007). Ayaad *et al*. (2001) recorded a significant THC increase in the last larval instar of the flesh fly *Parasarcophaga surcoufi*, at 40h post-injection with the LD_{30} and LD_{70} of Azt. Treatment of larvae of the tobacco cutworm *Spodoptera litura* with Azt enhanced the THC at 24 h post-treatment (Sharma *et al*., 2003). THC increased in the greater wax moth *Galleria mellonella* larvae after treatment with different doses of gibberellic acid (GA_3)(plant growth regulator, PGR) (Altuntaş *et al*., 2012). After application of essential oils of *Ocimum sanctum*, *Ocimum gratissimum* and *Ageratum conyzoides* onto the non-mulberry silkworm *Antheraea assama*, an initial increase of THC was recorded at early hours of treatment (Khanikor and Bora, 2012). The addition of indole-3-acetic acid (IAA, plant hormone of the auxin class) to a diet of larvae of the lesser wax moth *Achoria grisella* promoted THC, at all doses (Çelik *et al*., 2017).

For understanding the increase in THC after the treatment of some insects with different botanicals, some scenarios can be provided. The THC increase may be due to a defensive action of botanicals against the hemocyte detoxification (George, 1996; George and Ambrose, 2004). Also, an increase in THC has been proposed owing to the promotion of hematopoiesis (Kurt and Kayis, 2015) or the release of hemocytes that adhered on surfaces (sessile haemocytes) within the haemocoel (Ghasemi *et al*., 2013a). In addition, the increase of THC may be due to the activation of a mitotic division of haemocytes which has been activated in response to some plant extracts or plant products (Ratcliffe and George, 1976). Moreover, increasing THC can be regarded as an immune response of an insect against a pathogen or other foreign materials, such as the introduced plant extracts (Chu *et al*., 1993; Anderson *et al*., 1995; Ordas *et al*., 2000), since THC increase indicates that the hemocytes exhibit positive stress immunity in response to the tested botanical or toxic effect on the immunocytes (certain types of hemocytes) (Zibaee and Bandani, 2010a; Ghasemi *et al*., 2013b; Shaurub *et al*., 2014). It may be important to mention that the endocrine complex is involved in the haemocyte accumulation following some initial stimulus (Nappi, 1974). Early, Jones (1967b) suggested that ecdysteroids can regulate the

number of haemocytes. Because Azt can be considered as a responsible factor for the modification of haemolymph ecdysteroid titers (Redfern *et al*., 1982; Barnby and Klocke, 1990), some plant extracts, such as *Nigella sativa* extracts (Ghoneim *et al*., 2015d) may act as an antiecdysteroid materials enhancing the THC (see Table 1).

Table 1: Influenced THC (cell/mm^3) in nymphs and adults of *Schistocerca gregaria* by different extracts of *Nigella sativa.*

Extract	Conc.		Nymphal age			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	15.0	mean ± SD	4783.3 ± 152.8 d	7283.3 ± 208.1 c	3216.7 ± 104.1 d	2200.0 ± 150.0 d
		Change %	+87.6	+16.2	-49.5	-31.3
	7.5	mean ± SD	5800.0 ± 200.0 d	4533.3 ± 125.8 d	3033.3 ± 202.1 d	1383.3 ± 125.8 d
		Change %	+127.5	-27.7	-52.4	-56.8
Petroleum ether	15.0	mean ± SD	1850.0 ± 132.3 c	5983.3 ± 104.1 b	4666.7 ± 160.7 d	3166.7 ± 125.8 a
		Change %	-27.5	-4.5	-26.7	-1.0
	7.5	mean ± SD	5616.7 ± 125.8 d	6866.7 ± 104.1 b	8733.3 ± 104.1 d	2933.3 ± 125.8 a
		Change %	+120.3	+9.6	+37.2	-8.3
n-butanol	15.0	mean ± SD	2333.3 ± 152.8 a	17416.7 ± 175.6 d	5800.0 ± 180.3 b	916.7 ± 175.6 d
		Change %	-8.5	+177.9	-8.9	-71.4
	7.5	mean ± SD	2400.0 ± 150.0 a	6466.7 ± 125.8 a	5816.7 ± 175.6 b	3633.3 ± 152.8 b
		Change %	-5.9	+3.2	-8.6	+13.5
Control		mean ± SD	2550.0 ± 180.3	6266.7 ± 125.8	6366.7 ± 125.8	3200.0 ± 132.3

Conc.: Concentration levels (%), mean ± SD followed with the letter (a): not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). (After Ghoneim et al., 2015d).

2.2. Decreasing THC

On the other hand, decreasing THC had been reported in many insects by various plant extracts or plant-derived materials, such as the cotton bollworm *Helicoverpa armigera* by *Artemisia annua*, *A. conyzoides*, and *Azadirachta indica* oils (Padmaja and Rao, 2000); *S. litura* by *Acorus calamus* oils (Sharma *et al*., 2008); the lemon butterfly *Papilio demoleus* by leaf extracts of *Eucalyptus globules*, *A. conyzoides* and *Allium sativum* (Pandey *et al*., 2012); the red cotton stainer *Dysdercus cingulatus* after feeding of 5th instar larvae on fresh food treated with 50% crude leaf extract of *A. conyzoides* (Pandey *et al*., 2007), as well as *S. littoralis* by certain concentrations of some compounds derived from urea and rice straw (Hassan *et al*., 2013). After feeding the rice moth *Corcyra cephalonica* larvae on rice treated with powdered leaves of *Lantana camara*, *Clerodendrum inerme* and *Citrus limon*, there was a remarkable reduction in the THC (Morya *et al*., 2010). Treatment of the *E. integriceps* nymphs with *A. annua* extract resulted in a reduction of THC (Zibaee and Bandani, 2010a). THC of the Mediterranean flour moth *Ephestia kuehniella* was

significantly decreased by the increasing concentration of *Ferula gummosa* oil (Ghasemi *et al*., 2013b). After the treatment of the 6th instar larvae of *H. armigera* with aqueous leaf extract of *Clerodendron inerme*, THC was reduced (Kalyani and Holihosur, 2015). Injection of different concentrations of the abscisic acid (ABA) into the haemocoel of *G. mellonella* larvae caused a remarkable reduction of THC (Er and Keskin, 2016). In addition, remarkable reductions of THC in the haemolymph of adults of both the desert locust *Schistocerca gregaria* and the migratory locust *Locusta migratoria* were recorded after treatment with acetone extract of *Calotropis procera* (Kaidi *et al*., 2017). THC in larvae of *S. littoralis* was significantly regressed after 48 hours of treatment of the 4th instar larvae with LC_{25} of *C. procerae* and *Atriplex halimus* extracts (Asiri, 2017).

According to the currently available literature, Azt and some of its formulations considerably reduced the THC in many insect species, such as American cockroach *Periplaneta americana* (Qadri and Narsaiah, 1978), last instar female nymphs of the brown-spotted locust *Cyrtacanthacris tatarica* (John and Ananthackrishnan, 1995), *S. litura* (Sharma *et al*., 2003) and *Dysdercus cingulatus* (Pandey and Tiwari, 2011). Azambuja *et al.* (1991) and Azambuja and Garcia (1992) reported that the administration of Azt *via* a blood meal to the last nymphal instar of the kissing bug *Rhodnius prolixus* led to a remarkable reduction in the THC. Drastic reduction of THC in the red cotton bug *Dysdercus koenigii* (Saxena and Tikku, 1990); the African monarch *Danaus chrysippus* (Pandey *et al*., 2008) and *S. littoralis* (Shaurub *et al*., 2014) had been recorded after treatment with Azt. With regard to the Azt formulations, maintaining of the banana rhizome weevil *Cosmopolites sordidus* up to 96 hours on neem gold-treated banana rhizomes, THC was reduced (Sahayaraj and Kombiah, 2010). After topical application of NeemAzal onto the last instar larvae of *G. mellonella*, Er *et al*. (2017) recorded a sharp reduction of THC in haemolymph at 24 and 48 h post-treatment.

To interpret the THC reduction in insects by botanicals, it is important to point out that the hemocyte populations are influenced by the mitotic division of the circulating hemocytes (Gardiner and Strand, 2000; Er *et al*., 2010). For example, the number of mitotic hemocytes can be an acceptable explanation for reduced THC (Er *et al*., 2017). In some studies, the antimitotic effects and cell cycle arrest have been demonstrated by certain botanicals (Salehzadeh *et al*., 2003; Huang *et al*., 2011). In addition, the reduction of THC may be a result of the inhibition of larval hematopoietic function and cell proliferation (Zhu *et al*., 2012). As suggested by many authors (Sharma *et al*., 2003; Sabri and Tariq, 2004; Tiwari *et al*., 2006; Pandey *et al*., 2007; Sendi and Salehi, 2010; Zhu *et al*., 2012; Zibaee *et al*., 2012), reduction of THC may be attributed to the cytotoxicity of botanicals and the death of pathologically degenerated cells.

Few investigators have examined the effects of insect hormones on the hemocyte populations, such as detrimentally reduced THC in the haemolymph of *S. litura* larvae (Rao *et al*., 1984) and 5th instar nymphs of *D. cingulatus* (Ahmad, 1995) after treatment with β-ecdysone. Thus, it is possible to explain the decrease in THC by the inhibitory effects of botanicals on the endocrine organs (Sharma *et al*., 2003; Sabri and Tariq, 2004; Pandey *et al*., 2007; Zhu *et al*., 2012; Zibaee *et al*., 2012). However, some authors (Huang *et al*., 2011; Shu *et al*., 2015; Er *et al*., 2017) reported that Azt treatment led to apoptosis and autophagy in insect cell lines resulting in cell death. The reduction in THC after treatment with certain botanicals may be attributed to the nodulation, encapsulation and phagocytosis (Pandey *et al*., 2007) and/or their toxic effects on the immune cells (Sadeghi *et al*., 2017**)**.

2.3. Diverse or Contradictory Results of THC in the Same Insect:

Literature sources contain diverse or contradictory results of THC after treatment of the same insect with plant extracts or products, depending on some factors, such as the developmental stage, age and time of the treated insect, as well as the botanical

concentration and the polarity of the used solvents. For example, treatment of the beetle *Xanthogaleruca luteola* with *A. annua* extracts resulted in decreased THC at 6 and 12 hr post-treatment but increased THC 24 and 48 h (Kohan and Sendi, 2013). Ghoneim *et al*. (2015d) treated the penultimate (4^{th}) instar nymphs of *S. gregaria* with extracts of *Nigella sativa* seeds. In the early-aged last instar nymphs, THC remarkably increased by methanol extract of *N. sativa* seeds but slightly decreased by n-butanol extract. In mid-aged nymphs, only n-butanol extract promoted the hemocyte production, regardless the concentration, while the effects of other extracts depended on the concentration. In late-aged nymphs, all extracts exhibited inhibitory effects on THC (for some detail, see Table 1). As recorded by Sadeghi *et al*. (2017), treatment of the 4^{th} instar larvae of the corn stem borer *Sesamia cretica* with 1000 ppm of the essential oil of *Ferula ovina* promoted the THC, followed by a dose-dependent decrease of THC at 2500 and 7000 ppm. In the same insect, THC was variously affected by the essential oil since a decrease in THC was recorded until 12 hr but an increase was observed by 24-48 hr.

3. Differential Haemocyte Populations:

In insects, there are several types of hemocytes. The most common types are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adiphohaemocytes (ADs), coagulocytes (CGs) and oenocytoids (OEs). It is important to emphasize that not all of these hemocyte types exist in all insect species. Also, their characteristic features are slightly different in various insect species (for some detail, see Meister and Lagueux, 2003; Theopold *et al*., 2004; Kanost *et al*., 2004; Meister, 2004; Ribeiro and Brehelin, 2006; Lamprou *et al*., 2007; Wang *et al*., 2010; Manachini *et al*., 2011; Browne *et al*., 2013; Siddiqui and Al-Khalifa, 2014; Er *et al*., 2017).

3.1. Differential Haemocyte Populations in Normal Insects:

Depending on the literature sources, differential hemocyte counts (DHCs) of various types of circulating hemocytes depend on the insect life cycle, developmental stage, age, sex, nutrition, circadian rhythm and other factors. For example, Jones and Liu (1961) observed increasing PRs in the bug *R. prolixus* prior to ecdysis. At the time of ecdysis, PRs and GRs increased, whereas PLs, OEs and ADs decreased. After ecdysis, PLs and OEs were greater in number but GRs were fewer. Also, Jones (1967a) determined the DHC in *R. prolixus* and recorded an increase of PLs but a decrease of GRs during the fasting period following each moult. In the bollworm *Heliothes zea*, Shapiro *et al*. (1969) reported an increase of SPs from 38% in 5-day-old larvae to 59% in 8-day-old larvae and then there was a decrease in the number of these hemocytes. The PRs and PLs initially decreased from 5 to 8 days and then increased until pupation. However, the OEs remained stable at 1-2%. According to Nappi (**1970**), PLs were the major type of haemocytes during the development of the fly *Drosophila euronotus*. In the *D. cingulatus* adults, Zaidi and Khan (1975) recorded very little PRs% in comparison with that of PLs and ADs. In addition, OEs and GRs were poorly represented. Furthermore, PLs% was higher in the newly emerged adult females than in adult males of the same age. However, PLs count increased in both sexes with ageing, especially after the first reproductive cycle, whereas Ads count was the highest of all cell types and were significantly higher in the males than in females following emergence. Contrary to PLs, ADs count decreased with the age and was low in both sexes, especially after the first reproductive cycle. As reported by Dunphy and Nolan (1980), PLs and GRs were the predominant hemocyte types in both sexes of the spruce budworm *Choristoneura fumiferana*. PLs decreased but GRs increased throughout the larval development of both sexes. In all developmental stages, there were more PLs in the males than in the females. Also, there were more GRs in males than in females until the 6^{th} instar when the reverse occurred. Islam and Roy (1982) determined increasing PRs% and SPs%

in the Asian cricket *Schizodactylus monstrosus* over those of GRs, PLs and ADs, at night. Nishi (1982) observed increasing PLs% in *S. litura* from the 5th instar to late 6th instar during the larval stage (pre-pupal), reaching the maximal % of these cells which declined in 2-day-old pupae. In contrast, PRs% decreased at the prepupal phase as compared to those of PLs and PRs, which reached their maximal % in the pupal stage. The population density of ADs was the maximum at the prepupal phase. The SPs% was higher in 6th instar larvae than in 2-day-old 5th instar and late 6th instar larvae. In *S. litura* larvae, the proportions of GRs and PLs were 37-47% and 16-35% from the 5th molting through 6th instar, respectively (Kurihara *et al*., 1992).

In the present century, Gardiner and Strand (2000) reported that PLs were the most abundant hemocyte type during the early larval instars, while GRs were the most abundant type during the last instar of the soybean looper *Chrysodeixis includes* and the fall armyworm *Spodoptera frugiperda*. In *P. demoleus*, PR population remains low throughout the larval stage, except in the 1st instar where their % was high. Their population declined in the latter instars but GRs% increased (Jalali and Salehi (2008). PLs were the most abundant hemocytes in the red palm weevil *Rhynchophorus ferrugineus* larvae, the ground beetle *Carabus lefebvrei* (Giglio *et al*., 2008), and in the eusocial stingless bee *Melipona scutellaris* (Amaral *et al*., 2010). In the 6th (last) instar larvae of the lawn armyworm *Spodoptera mauritia*, the number of PLs was the highest among different hemocyte types (Manogem *et al*., 2016). The DHC of each hemocyte type (GRs, OEs, PLs and SPs) in the vine moth *Eupoecilia ambiguella* varied among diets (Vogelweith *et al*., 2016). In normal larvae of the tenebrionid beetle *Platynotus belli*, Chavan *et al*. (2017) estimated GRs count as the highest type, followed by PRs, ADs, OEs, PLs, CGs and SPs, respectively. Ghoneim *et al*. (2017) determined ADs as the largest count in haemolymph of full-grown larvae of the pink bollworm *Pectinophora gossypiella*, followed by other hemocyte types, regardless the age. Also, this hemocyte type considerably increased with the larval age. On the other hand, the least hemocyte population was estimated for OEs, regardless the age. In addition, OEs slightly increased with the larval age. In contrast, other hemocyte types decreased with the age of larvae.

It may be important to point out that the increasing counts of certain haemocyte types and decreasing counts of other types may be due to the transformation of some types into other ones for achieving the phagocytic function or other tasks in the battle against the biotic targets, like bacteria, yeast and apoptic bodies and abiotic materials, such as particles of Indian ink or chemical constitutions of the plant extracts (Hernandez *et al*., 1999; de Silva *et al*., 2000).

3.2. Differential haemocyte populations as affected by botanicals

The available literature has been enriched with many reported results about the DHC alterations in various insects as a response to the treatment with botanicals. For example, feeding of *D. chrysippus* 5th instar larvae for 24 hr on fresh food treated with the crude leaf extract of *A. conyzoides* led to some differences in the hemocyte profile as PRs, PLs and GRs decreasing, SPs, ADs and OEs increasing, in both groups of larvae after 24 and 48 hr post-treatment (Pandey *et al*., 2007). After oral administration of sweet flag rhizome oil (*A. calamus*) into the last (6th) instar larvae of *S. litura*, Sharma *et al*. (2008) recorded decreases in the populations of PRs, PLs and SPs and increases in the populations of GRs and OEs, after 24-72 hr of treatment. Also, Er *et al*. (2017) recorded a significant reduction of GRs but an increase of PLs in larvae of *G. mellonella* only after treatment with 100 ppm Azt, whereas no difference was observed in PRs and OEs ratios. Shaurub and Sabbour (2017) investigated the impact of acetone extract of *M. azedarach* fruits on the haemolymph picture of the last instar larvae of *A. ipsilon*. They recorded increasing counts of PLs and GRs, but decreasing PRs, SPs and OEs. Çelik *et al*. (2017) evaluated the effect

of IAA (PGR of auxin class) on hemocytes of *A. grisella*. Depending on their results, PLs% decreased but GRs% increased. Nevertheless, the percentages of SPs, PRs and OEs did not alter.

In the context of fluctuated DHCs in different insects by various botanicals, more attention should be paid specifically for certain hemocyte types herein. With regard to PLs population, its enhancement was reported in larval haemolymph of some insects, such as *S. littoralis* after treatment with some urea compounds derived from urea waste or rice straw (Hassan *et al*., 2013) and LC_{25} of *C. procerae* and *A. halimus* extracts (Asiri, 2017). Er and Keskin (2016) injected different concentrations of ABA into the haemocoel of *G. mellonella* larvae and observed a remarkable alteration in PLs ratio, in a dose-dependent manner, at different time intervals. After topical application of NeemAzal (commercial Azt formulation) onto the last instar larvae of the same insect, Er *et al*. (2017) recorded a significant increase of PLs, only at 100 ppm.

On the contrary, treatment of nymphs of *E. integriceps* and *R. prolixus* with Azt and *A. annua* extracts resulted in a dose-dependent decrease of PLs count (Azambuja *et al*., 1991; Zibaee and Bandani, 2010 a, b). PLs population was declined in some insects by some plant extracts, such *S. littoralis* by LC_{25} or LC_{50} of Azt (Rizk *et al*., 2001), *S. gregaria* by Spinosad (bacteria-based product) (Halawa *et al*., 2007) as well as *C. tatarica* (John and Ananthackrishnan, 1995) and *P. surcoufi* (Ayaad *et al*., 2001) by Azt. The fluctuation of hemocyte counts in the immune challenged *S. litura* was found to cause a decline of PLs in haemolymph, at 24-72 hr post-treatment with the *A. calamus* essential oil (Sharma *et al*., 2008). PLs population in the 4th instar larvae of *Cx, quinquefasciatus* had been remarkably reduced after 48 hrs of treatment with LC_{50} values of bark essential oil of *Cinnamomum osmophloeum* and the leaf essential oil of *Matricharia chamomella* (Gad and El-DaKheel, 2009). A similar reduction of PLs was recorded in the 2nd instar larvae of *Culex pipiens* after treatment with methanolic extracts of *Solanum nigrum*, *Acokanthera spectabilis,* and *Heliotropium aegyptiacum* (Zahran and Gad, 2013). Treatment of penultimate instar nymphs of *S. gregaria* with different extracts of *N. sativa* seeds resulted in a reduction of PLs count in haemolymph of last instar nymphs and newly emerged adult females (Ghoneim *et al*., 2015d, for some detail, see Tables 2-4). After the injection of four doses of neem essential oil into the *G. mellonella* larvae, a significant decrease was recorded for PLs count in the haemolymph of larvae (Haszcz, 2016). In addition, treatment of diet of the *A. grisella* larvae with different doses of IAA resulted in a decrease of PLs (Çelik *et al*., 2017). Treatment of the 4th instar larvae of *S. cretica* with 1000 ppm of *F. ovina* essential oil led to a dose-dependent reduction of PLs count after 48 hr of treatment (Sadeghi *et al*., 2017).

For the interpretation of PLs reduction in insects, after treatment with certain botanicals, some suggestions may be conceivable. The reduction of PLs count in some insects may be due to the transformation of a number of this cell type into other types (George, 1996). In other words, the decreasing PLs count can be attributed to the fact that these hemocytes are highly polymorphic and can be converted into other haemocyte types (Gupta and Sutherland, 1966). On the other hand, some botanicals may prohibit the hematopoietic organs that are responsible for the production of PLs (Tiwari *et al*., 2002). In contrast, the increase in PLs population can be attributed to the differentiation of haemocytes by mitosis (Kurihara *et al*., 1992). The role of PLs in phagocytosis is disputed because some authors believed that they are phagocytes (Tojo *et al*., 2000; Ling and Yu, 2006) while other authors reported no phagocytic function (Beaulaton, 1979). In this respect, the transformation of PLs for cellular defence by loss of a portion of cytoplasm or by fragmentation or by gradual rounding off of fusciform PLs results in a reduction in the PLs population (Pathak and Kulshreshtha, 1993).

With regard to the GRs, the increasing count was reported in *S. littoralis* as a response to Margosan-O (commercial Azt preparation) (Rizk *et al*., 2001). The immune-challenged *S. litura* larvae by the essential oil of *A. calamus* resulted in an enhancement of GRs in haemolymph, at 24-72 h post-treatment (Sharma *et al*., 2008). Feeding of the *A. grisella* larvae on a diet supplemented with IAA (PGR of auxin class) led to increases of GRs, at 2, 5, 100, 200 and 1,000 ppm (Çelik *et al*., 2017). Shaurub and Sabbour (2017) recorded a significant increase in GRs population in the last instar larvae of *A. ipsilon* after treatment with acetone extract of *M. azedarach* fruits.

Table 2: Influenced DHC (%) in nymphs and adults of *S. gregaria* by methanol extract of *N. sativa*.

Developmental stage		Conc.		PLs	GRs	CGs
Nymphal age	Early-aged	15.0	mean ± SD	12.3 ± 1.5 d	35.0 ± 1.0 d	52.7 ± 2.5 c
			change %	-67.4	+50.2	+32.7
		7.5	mean ± SD	13.0 ± 1.7 d	28.3 ± 2.5 b	57.0 ± 3.0 c
			change %	-65.5	+21.5	+43.6
		Control	mean ± SD	37.7 ± 1.2	23.3 ± 1.2	39.7 ± 2.3
	Mid-aged	15.0	mean ± SD	13.7 ± 1.2 d	28.7 ± 1.5 c	57.7 ± 2.5 d
			change %	-49.3	-20.3	+55.9
		7.5	mean ± SD	6.3 ± 0.6 d	48.7 ± 1.2 d	45.7 ± 1.5 c
			change %	-76.7	+35.3	+23.5
		Control	mean ± SD	27.0 ± 2.0	36.0 ± 1.0	37.0 ± 1.0
	Late-aged	15.0	mean ± SD	4.0 ± 2.1 a	54.7 ± 2.1 d	41.3 ± 3.1 d
			change %	-24.5	+95.4	-38.1
		7.5	mean ± SD	4.7 ± 2.9 a	53.7 ± 1.5 d	41.7 ± 1.5 d
			change %	-11.3	+91.8	-37.5
		Control	mean ± SD	5.3 ± 1.5	28.0 ± 1.0	66.7 ± 2.3
Newly emerged adults		15.0	mean ± SD	3.3 ± 1.5 d	62.7 ± 2.5 c	34.0 ± 1.7 c
			change %	-85.7	+22.9	+30.8
		7.5	mean ± SD	11.7 ± 1.5 d	56.0 ± 1.7 b	32.3 ± 2.5 b
			change %	-49.1	+9.8	+24.2
		Control	mean ± SD	23.0 ± 1.7	51.0 ± 2.0	26.0 ± 1.0

Conc., a, b, c, d: see footnote of Table (1). PLs: plasmatocytes, GRs: granulocytes, CGs: coagulocytes. (After Ghoneim et al., 2015d) .

Table 3: Influenced DHC (%) in nymphs and adults of *S. gregaria* by petroleum ether extract of *N. sativa*.

Developmental stage		Conc.		PLs	GRs	CGs
Nymphal age	Early-aged	15.0	mean ± SD	16.7 ± 1.5 d	49.3 ± 2.5 d	34.0 ± 1.0 b
			change %	-55.7	+111.6	-14.4
		7.5	mean ± SD	5.7 ± 1.5 d	61.3 ± 1.5 d	34.3 ± 2.1 b
			change %	-84.9	+163.1	-13.6
		Control	mean ± SD	37.7 ± 1.2	23.3 ± 1.2	39.7 ± 2.3
	Mid-aged	15.0	mean ± SD	12.3 ± 1.5 d	41.0 ± 1.0 c	59.7 ± 2.5 d
			change %	-54.4	+13.9	+61.4
		7.5	mean ± SD	5.0 ± 1.0 d	40.0 ± 1.0 c	55.7 ± 1.5 d
			change %	-81.5	+11.1	+50.5
		Control	mean ± SD	27.0 ± 2.0	36.0 ± 1.0	37.0 ± 1.0
	Late-aged	15.0	mean ± SD	2.0 ± 1.7 a	58.0 ± 1.0 d	40.0 ± 1.0 d
			change %	-62.3	+107.1	-40.0
		7.5	mean ± SD	2.7 ± 1.2 a	53.7 ± 1.5 d	43.7 ± 2.5 d
			change %	-49.1	+91.8	-34.5
		Control	mean ± SD	5.3 ± 1.5	28.0 ± 1.0	66.7 ± 2.3
Newly emerged adults		15.0	mean ± SD	9.3 ± 1.5 d	51.3 ± 0.6 a	39.3 ± 1.5 d
			change %	-59.6	+0.6	+51.2
		7.5	mean ± SD	10.3 ± 2.5 c	52.0 ± 1.7 a	37.7 ± 1.2 d
			change %	-55.2	+2.0	+45.0
		Control	mean ± SD	23.0 ± 1.7	51.0 ± 2.0	26.0 ± 1.0

Conc., a, b, c, d: see footnote of Table (1). PLs, GRs, CGs: see footnote of Table (2). (After Ghoneim et al., 2015d)

In contrast, the GRs count was reduced in the fly *P. surcoufi* after treatment with Azt (Ayaad *et al*., 2001) and in the *S. littoralis* larvae after treatment with some compounds derived from urea waste and rice straw (Hassan *et al*., 2013). The number of GRs decreased in nymphs of both bugs *E. integriceps* and *R. prolixus* after treatment with Azt and *A. annua* extracts, in a dose-dependent course (Azambuja *et al*., 1991; Zibaee and Bandani, 2010 a, b). After treatment of the 4th instar larvae of *Cx, quinquefasciatus* with LC_{50} values of the bark essential oil of *C. osmophloeum* and the leaf essential oil of *M. chamomella*, GRs had

been remarkably reduced at 48 hrs post-treatment (Gad and El-DaKheel, 2009). A similar result was recorded after-treatment of the 2nd instar larvae of *Cx. pipiens* with methanolic extracts of *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* (Zahran and Gad, 2013). In addition, treatment of the 4th instar larvae of *S. cretica* with 1000 ppm of essential oil of *F. ovina* resulted in a reduction of GRs count (Sadeghi *et al*., 2017). After 48 hrs of treatment of *S. littoralis* 4th instar larvae with LC_{25} of *C. procerae* and *A. halimus* extracts, GRs count decreased in the larvae (Asiri, 2017).

Table 4: Influenced DHC (%) in nymphs and adults of *S. gregaria* by n-butanol extract of *N. sativa*.

Developmental stage		Conc.		PLs	GRs	CGs
Nymphal age	Early-aged	15.0	mean ± SD	16.3 ± 0.6 d	57.0 ± 1.7 d	56.0 ± 1.7 c
			change %	-56.8	+144.6	+41.1
		7.5	mean ± SD	8.7 ± 2.1 d	35.3 ± 1.5 d	56.0 ± 1.0 d
			change %	-76.9	+51.5	+41.1
		Control	mean ± SD	37.7 ± 1.2	23.3 ± 1.2	39.7 ± 2.3
	Mid-aged	15.0	mean ± SD	7.0 ± 1.0 d	41.3 ± 1.5 c	51.7 ± 2.5 d
			change %	-74.1	+14.7	+39.7
		7.5	mean ± SD	12.7 ± 0.6 d	37.7 ± 0.6 a	49.7 ± 0.6 d
			change %	-53	+4.7	+34.3
		Control	mean ± SD	27.0 ± 2.0	36.0 ± 1.0	37.0 ± 1.0
	Late-aged	15.0	mean ± SD	4.3 ± 1.5 a	68.4 ± 2.9 d	27.3 ± 1.5 d
			change %	-18.9	+144.3	-59.1
		7.5	mean ± SD	4.7 ± 1.5 a	59.7 ± 2.3 d	35.7 ± 1.2 d
			change %	-11.3	+113.2	-46.5
		Control	mean ± SD	5.3 ± 1.5	28.0 ± 1.0	66.7 ± 2.3
Newly emerged adults		15.0	mean ± SD	4.7 ± 2.1 d	42.3 ± 1.5 c	53.0 ± 1.0 d
			change %	-79.6	-17.1	+103.8
		7.5	mean ± SD	21.0 ± 1.0 a	39.7 ± 0.6 d	39.3 ± 1.2 d
			change %	-8.7	-22.2	+51.2
		Control	mean ± SD	23.0 ± 1.7	51.0 ± 2.0	26.0 ± 1.0

Conc., a, c, d: see footnote of Table (1). PLs, GRs, CGs: see footnote of Table (2). (After Ghoneim et al., 2015d)

Moreover, increasing or decreasing of GRs in haemolymph depends upon the concentration of the tested botanical and/or the developmental stage of the same insect. For example, Ghoneim *et al*. (2015d) recorded that both methanol and petroleum ether extracts of *N. sativa* seeds generally enhanced the GRs population in last instar nymphs and newly emerged adult females of *S. gregaria*. Also, a similar promoting effect was exhibited by n-butanol extract on the same hemocyte type only in nymphs but its count was exceptionally regressed in adults. Sadeghi *et al*. (2017) treated the 4th instar larvae of *S. cretica* with some concentrations of the essential oil of *F. ovina* and recorded an increase of GR count at 1000 ppm but GR decreases at 2500 and 7000 ppm. Among 100, 1000 and 3000 ppm of NeemAzal, Er *et al*. (2017) recorded a significant reduction in GRs count only after topical application of 100 ppm onto the last instar larvae of *G. mellonella*.

The increasing count of GRs may be explained by the transformation of some haemocytes (such as PLs and PRs) into GRs (Gupta and Sutherland, 1966) which reveals the role of the latter hemocytes in the detoxification of toxic components in the plant extracts (Kurihara *et al*., 1992; George and Ambrose, 2004). In their study on the *A. ipsilon* larvae, Shaurub and Sabbour (2017) concluded that the acetone extract of *M. azedarach* fruits might stimulate the cellular immune system of larvae *via* an increasing number of the phagocytic GRs. On the other hand, reduction in the GRs count may be due to the transformation of these haemocytes into cystocytes or OEs by extension of some cytoplasmic granules along the inner periphery of the nuclear membrane (Gupta and Sutherland, 1966). One of the main functions of GRs is phagocytosis of the foreign bodies, as reported in *P. gossypiella* (Raina, 1976); the mulberry silkworm *Bombyx mori* (Wago, 1980); *G. mellonella* (Tojo *et al*., 2000); the tobacco hornworm *Manduca sexta* (Nardi *et al*., 2001); *H. armigera* (Essawy *et al*., 1985); the beet armyworm *Spodoptera exigua* (Pendland and Boucias, 1996); the gypsy moth *Lymantria dispar* (Butt and Shields, 1996); and *S. littoralis* (Costa *et al*., 2005**)**. Therefore, decreasing of the observable GRs population may be due to the destruction of some number of these cells during this immune reaction against the tested botanicals.

In connection with PRs, their count had been remarkably reduced after 48 hr of treatment of the 4th instar larvae of *Cx, quinquefasciatus* with LC_{50} values of the bark essential oil of *C. osmophloeum* and the leaf essential oil of *M. chamomella* (Gad and El-DaKheel, 2009). A similar result was recorded after treatment of the 2nd instar larvae of *Cx. pipiens* with the methanolic extract of *S. nigrum, A. spectabilis,* or *H. aegyptiacum* (Zahran and Gad, 2013). After 48 hrs of treatment of *S. littoralis* 4th instar larvae with LC_{25} of *C. procerae* and *A. halimus* extracts, PRs count was declined in haemolymph of larvae (Asiri, 2017). After topical treatment of NeemAzal onto the last instar larvae of *G. mellonella,* Er *et al*. (2017) recorded no difference in PRs population. It is interesting to point out that the changes in PRs population may be attributed to some factors including inhibition of their mitotic division, their conversion to other cell types, or to the inhibition of the activity of hematopoeitic organs responsible for their production (Pandey *et al*., 2012).

In respect of the OEs, their count had been remarkably enhanced after 48 hr of treatment of *Cx, quinquefasciatus* 4th instar larvae with LC_{50} values of the bark essential oil of *C. osmophloeum* and the leaf essential oil of *M. chamomella* (Gad and El-DaKheel, 2009). A similar result was reported after treatment of *Cx. pipiens* 2nd instar larvae with the methanolic extract of *S. nigrum, A. spectabilis* or *H. aegyptiacum* (Zahran and Gad, 2013). Shaurub and Sabbour (2017) reported that the number of OEs significantly increased in the last instar larvae of *A. ipsilon* after treatment with acetone extract of *M. azedarach* fruits. After 48 hours of treatment of *S. littoralis* 4th instar larvae with LC_{25} of *C. procerae* or *A. halimus* extracts, OEs count increased in larvae (Asiri, 2017). After topical treatment of

NeemAzal onto last instar larvae of *G. mellonella*, Er *et al*. (2017) found no difference in DHCs of OEs, regardless of the concentration. The addition of IAA to the diet of *A. grisella* larvae did not affect the OEs population (Çelik *et al*., 2017).

It is believed that OEs play an important role in phenoloxidase (PO) cascade when an immune challenge occurs (Beckage, 2008; Strand, 2008). Therefore, the significant increase in their population in *Ephestia kuehniella* larvae could be led to the stimulation of the immune system of the plant oil-treated larvae to the secretion of PO (Ghasemi *et al*., 2013b). According to Kurihara *et al*. (1992), the induced increase in the OEs count reveals their defensive function but the reduction in their numbers may be attributed to their transformation.

With regard to the CGs, Ghoneim *et al*. (2015d) could characterize this hemocyte type in the nymphs and adults of *S. gregaria*. Both methanol and n-butanol extracts of *N. sativa* in adults and the majority of nymphs considerably promoted CGs population. On the other hand, petroleum ether extract enhanced the CGs population in adults but tremendously prohibited it in the majority of nymphs. Therefore, the predominant increasing CGs count might be attributed to their role in phagocytosis (Brehelin and Hoffmann, 1980). In addition, the number of SPs significantly increased in the last instar larvae of *A. ipsilon* after treatment with acetone extract of *M. azedarach* fruits (Shaurub and Sabbour, 2017).

4. Cytopathological Effects of Botanicals on The Qualitative Haemocyte Profile in Insects:

Early, Jones and Tauber (1954) proposed a scheme for classifying the abnormal hemocytes, including grossly abnormal nuclear changes (such as extrusion of nuclear material, nuclear hypertrophy and vacuolation, pycnosis (atrophy), karyorrhexis, denucleation), grossly abnormal cytoplasmic changes (such as abnormal staining of granules or other inclusions, abnormal sizes of granules or other inclusions, gross vacuolation), abnormal changes in shape, volume, distribution or configuration of hemocytes (such as microhemocytes, poikilohemocytes, agglutination of cells). However, many abnormal changes in the insect hemocytes have been reported as responses to different stresses (Arvy *et al*., 1950; Arnold, 1952; Morgenthaler, 1953; Patton and Sarkaria, 1958).

Despite the botanicals represent a source of non-toxic compounds utilized for insect pest control, different pathological effects of botanicals on the hemocytes (morphological and histological characteristics) have been described only in a few insect species, such as the cockroach *P. americana* (Qadri and Narsaiah, 1978), the bug *D. koenigii* (Saxena and Tikku, 1990; Tikku *et al*., 1992), and the lepidopteran *S. litura* (Sharma *et al*., 2001, 2003, 2008). An earlier study was conducted by Shull *et al*. (1932) who observed granulation in the cytoplasm of haemocytes of the darkling beetle *Adesmia cancellata* after treatment with limonine and D-camphor. After treatment of the last instar female nymphs of *C. tatarica* with Azt, John and Ananthakrishnan (1995) observed bulging of cytoplasm, membrane destruction and release of cytoplasmic materials in PLs, while GRs showed vacuoles in the cytoplasm and nucleus. After treatment of *S. littoralis* larvae with Azt or its preparation Margosan-O, Rizk (1991) could not observe vacuolation in the cytoplasm of PLs. Bulging of some PLs and lysis of other hemocytes were caused by Azt in the last instar larvae of *P. surcoufi* (Ayaad *et al*., 2001). After treatment of *S. frugiperda* larvae with Azt, the scanning electron microscope (SEM) examination revealed that the *S. frugiperda* cells (SF-9 cells) displayed an increase in cell swelling and cell abnormalities after 48 hr of treatment, as low as 0.10 µg/ml Azt (Reed and Majumdar, 1998). Studies by SEM demonstrated the complete loss of filopods in PLs and cytoplasmic projections in GRs of *S. litura* larvae after treatment

with Neem gold (Sharma *et al*., 2003). Sharma *et al*. (2008) recorded similar effects of essential oil of *A. calamus* rhizomes on the larval hemocytes of *S. litura* as loss of cytoplasmic projections in GRs. Interestingly; they observed vacuolization in cytoplasm and degeneration of organelles, both in PLs and GRs. After treatment of *S. littoralis* 4th instar larvae with LC_{50} of Azt, Shaurub *et al*. (2014) observed several disorders of hemocytes, such as the presence of rough endoplasmic reticulum filled with fibrous materials in their cisternae, disorganization of mitochondria, and the cytoplasm was vacuolated with the appearance of autophagic lysosomes. In connection with the morphological disorders of hemocytes in *S. gregaria* by seed extracts of *N. sativa*, Ghoneim *et al*. (2015d) recorded that some GRs were lysed or appeared as small darkly stained cells after treatment with petroleum ether extract (for some detail, see Fig.s 1& 2). According to this study, also, some CGs had degenerated and others appeared with destroyed membranes and extruded cytoplasmic contents, regardless of the extract. The same authors observed, also, numerous vacuoles in the nuclei of some PLs by n-butanol extract and similar vacuoles appeared in the cytoplasm of some GRs by petroleum ether extract. These morphological disorders of hemocytes may be attributed to the disruptive action of certain chemical constituents of these seed extracts of *N. sativa*, such as thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, damascenine, tannins, flavonoids, saponins and alkaloids (Al-Ghamdi, 2001; Ali and Blunden, 2003; Sharma *et al*., 2009; Ali *et al*., 2012). On the other hand, plant extracts at the sub-lethal levels may 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 interferes with normal hemocyte function (Vey *et al*., 2002). As concluded by Anunradha and Annadurai (2008), Azt or other plant products may exert their activities on some haemocytes by targeting 'actin' which localized in the lamellar extensions of the cells, as interpreted for *Drosophila melanogaster*, *S. litura* and *Plutella xylostella*. The question of whether the hemocytes are affected directly or *via* some physiological or endocrinological pathway is yet to be answered in spite of reports that developmental effects caused by botanicals, such as Azt, were attributed to the disruption of endocrine events (Schmutterer, 1990 a, b).

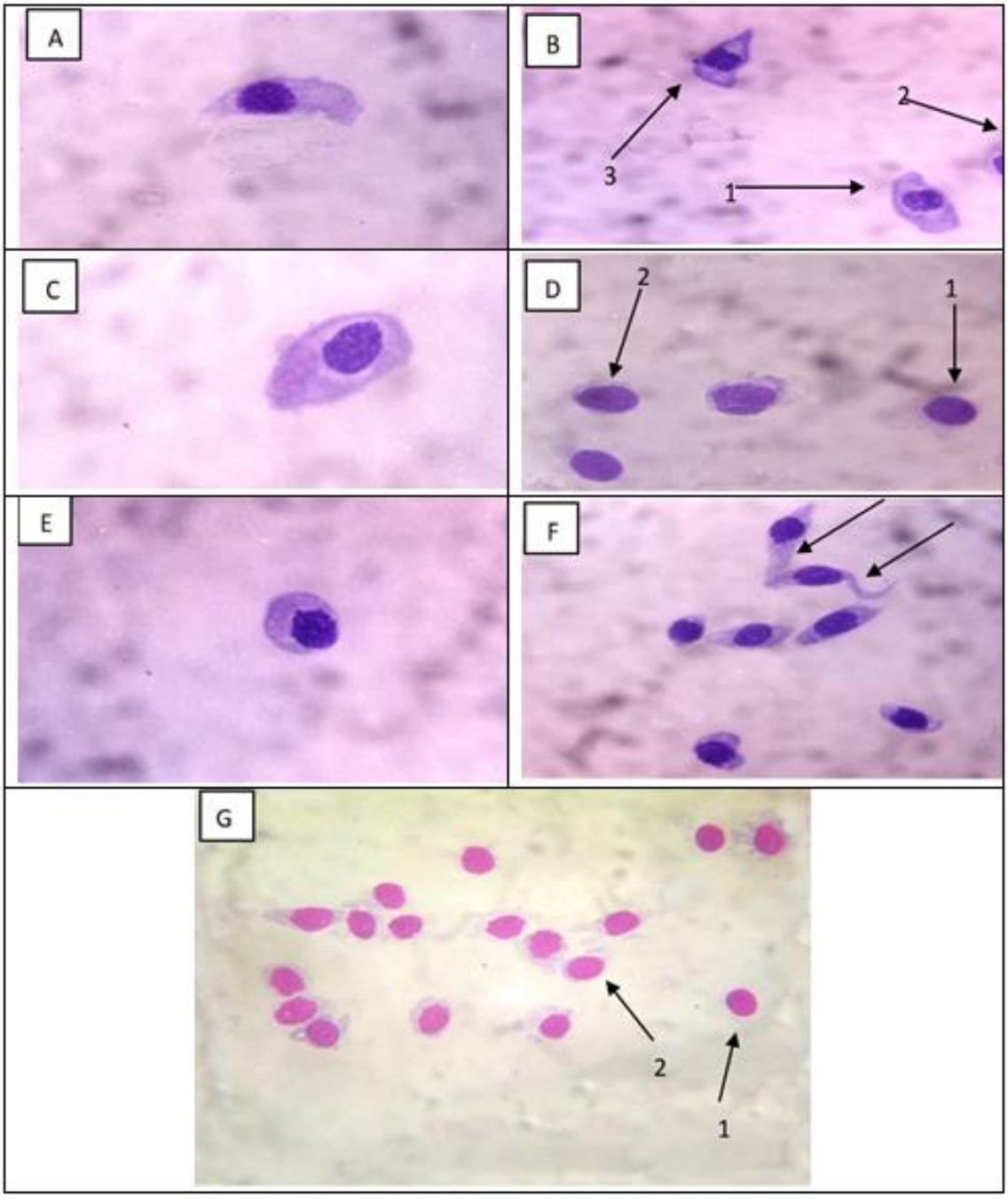

Fig. 1: Photomicrographs of normal circulating hemocytes of *S. gregaria*. (A): Spindle-shaped PLs with an eccentric nucleus. (B) 1: oval-shaped PLs with a centric nucleus, 2: round-shaped PLs with an eccentric nucleus, 3: spindle-shaped PLs with a centric nucleus. (C): Oval-shaped PLs with an eccentric nucleus. (D) 1: round-shaped GRs, 2: oval-shaped GRs. (E): Round-shaped GRs with a clear eccentric nucleus and without granules. (F): GRs with fillopodia. (G) 1: round-shaped CGs, 2: oval-shaped CGs. (After Ghoneim *et al.*, 2015d).

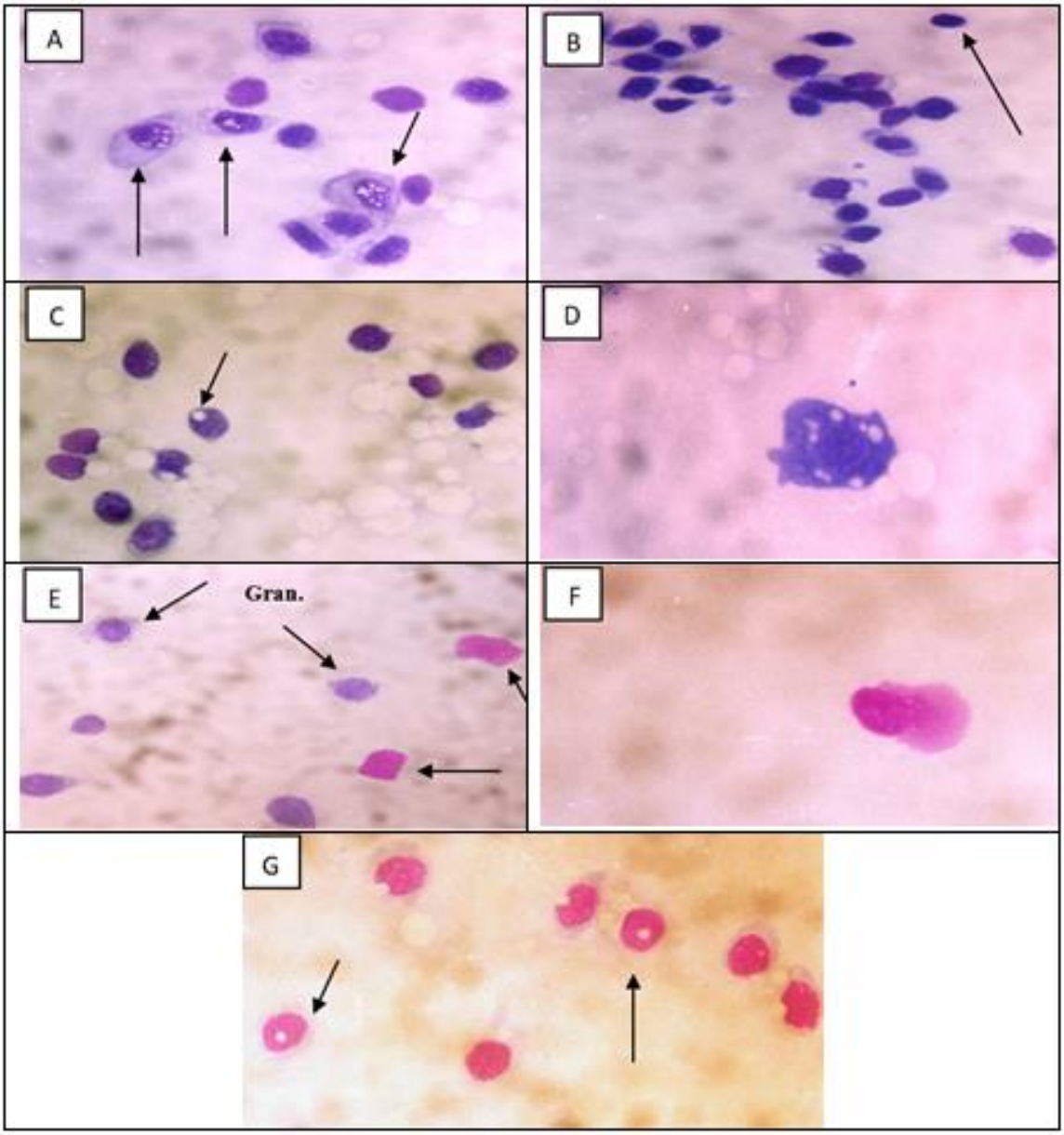

Fig. 2: Photomicrographs of deformed haemocytes in *S. gregaria* as a response to extracts of *N. sativa*. (A): PLs with vacuoles in the nucleus after treatment with the n-butanol extract. (B): small darkened GRs after treatment with methanol and petroleum ether extracts. (C): GRs contained vacuole after treatment with petroleum ether extract. (D): Lysed GRs with vacuoles after treatment with petroleum ether extract. (E): lysed GRs and CGs, regardless of the extract. (F): Destroyed cell membrane and extruded cytoplasmic contents in CGs, regardless of the extract. (G): CGs with vacuolated cytoplasm, regardless of the extract. (After Ghoneim *et al.*, 2015d).

5. Other Parameters of Insect Haemogram As Affected by Botanicals:

5.1. Fluctuated blood volume by botanicals

Characterization of total haemogram in insects includes, also, the determination of blood volume (BV) or haemolymph volume (HV) because the population of circulating hemocytes depends upon BV or is affected by it (Chapman, 1982; Bardoloi *et al*., 2016; Khosravi *et al*., 2016). In other words, BV determination is necessary in many cases for the accurate evaluation of THC (for some detail, see Ghoneim, 2019). As far as our literature survey could ascertain, no information was available about the effects of botanicals on BV in insects, except that study of John and Ananthakrishnan (1995). In this study, injection of Azt (2, 4 and 6 µl/g bodyweights of the test insect) into the haemocoele of last instar female nymphs of the locust *C. tatarica* led to significantly increased BV. A dose-dependent inverse relationship was recorded between the BV and THC.

5.2. Influenced Mitotic Index by Botanicals:

As reported by many authors (Gardiner and Strand, 2000; Tu *et al*., 2002; Saito and Iwabuchi, 2003; Okazaki *et al*., 2006), the maintenance of hemocyte populations in insects is thought to be regulated by mitotic division of the circulating hemocytes and by production/release of hemocytes in the hematopoietic organs. Mitotic index (MI) is a measure for the proliferation status of a hemocyte population and can be defined as the ratio between the numbers of cells undergoing mitosis to the THC in a population'. The MI is used as a criterion of response to various treatments involving factors that may affect this activity (for detail, see Ghoneim, 2019).

Some botanicals promote or inhibit MI in some insects, at certain concentrations and at specific times post-treatment. For example, Reed and Majumdar (1998) examined the influence of Azt on the proliferation of *S. frugiperda* cells (SF-9). Cell kinetic studies conducted at 24 hr intervals for 144 hr showed that Azt inhibited the cell multiplication of SF-9 cells, in a dose-dependent course. The antimitotic effects and cell cycle arrest of Azt have been demonstrated in insect cell lines in other studies (Salehzadeh *et al*., 2003; Huang *et al*., 2011). In agreement with these findings, Azt application onto *G. mellonella* resulted in a decrease of the mitotic hemocytes (reduced MI), which could be a possible explanation for reduced THC (Er *et al*., 2017). Altuntaş *et al*. (2012) recorded an increasing MI of hemocytes in *G. mellonella* larvae, at 5,000 ppm of GA_3. On the contrary, Er and Keskin (2016) observed a decrease in MI of hemocytes in larvae of the same insect, at 24 h post-injection of 10 and 50 mg/ml of ABA into the haemocoel. Moreover, the addition of ABA to the diet of *A. grisella* larvae did not affect MI of hemocytes (Çelik *et al*., 2017).

5.3. Affected Heartbeat by Botanicals:

Proper functioning of the heart is one of the prerequisite physiological processes for the insects (Feliciano *et al*., 2011) since a slight alteration in the heart functioning may interrupt the homeostasis and cause severe hazards in the insect physiology (Piazza and Wessells, 2011). Heartbeat rate (the number of beats per minute) was found to depend on different factors (for some detail, see Ghoneim, 2019). Depending on the currently available literature, studies of the effects of natural products on insect heart activity are scarce. Early, Orser and Brown (1951) investigated the effects of nine insecticidal products upon the rate of the heartbeat of *P. americana* after injection of different dosages into adult males. Among these products, rotenone steadily depressed the rate of heartbeat until it stopped. Despite the *M. azedarach* extracts and their limonoids exhibit various biological activities against insects, their effects on the insect heart activity were examined only in very few studies. As recorded by Ntalli *et al*. (2014), the application of methanolic fruit extract of *M. azedarach* and its limonoids onto the 5^{th} instar larvae of *S. exigua* showed no significant effects on the heart contraction frequency in larvae but the heartbeat frequency was remarkably decreased only in the 1-day old pupae. In this context, a comprehensive

study was conducted by Marciniak *et al*. (2010). They used the semi-isolated heart bioassay to evaluate the effect of glycoalkaloids (extracted from potato *Solanum tuberosum* leaves) on the heart contractile activity of three beetle species: the giant mealworm beetle *Zophobas atratus*, the mealworm beetle *Tenebrio molitor* and Colorado potato beetle *Leptinotarsa decemlineata*. After application of glycoalkaloids on the continuously perfused *Z. atratus*, the heart progressively inhibited the contraction frequency; higher concentrations exerted short and reversible cardiac arrests. In the other two beetles, glycoalkaloids failed to exhibit a cardiotropic effect. *In vivo* bioassay with 1-day old *Z. atratus* pupae showed that the phytochemical induced a negative inotropic effect on the heart.

6. Immune Responses and Defense Reactions of Insects Against Botanicals:

6.1. General Outline:

As reported by many authors (Hoffmann *et al*., 1996; Janeway and Medzhitov, 2002; Hoffmann, 2003; Strand, 2008; Berger and Jurcova, 2012), a well-developed immune system has been characterized in vertebrates for protection against various pathogenic organisms. This immune system is generally classified into two subsystems, innate and acquired immunity. Innate immunity involves a generic recognition and response to foreign invaders that is only temporary. On the other hand, the acquired immunity consists of specialized cells that identify specific agents and ultimately produce an immunological memory. Insects appear to lack the acquired immune response characteristic of the vertebrates. Therefore, insects rely on their highly efficient innate immunity for defense against pathogens and other invaders. The innate immune components of insects involve cellular and humoral defence mechanisms.

The physically defensive barriers in insects include the integument and the peritrophic membrane. A single layer of cells covered by a multilayered cuticle forms the integument, the outer surface of an insect body (Ashida and Brey, 1995). The peritrophic membrane is a layer made of chitin and glycoprotein that covers also the insect midgut. This membrane functions as a physical barrier against abrasive food particles and digestive pathogens (Hegedus *et al*., 2009). These structures constitute the initial protection for the haemocoele (the insect body cavity) and the midgut epithelium against invading microorganisms. When the invading microorganisms enter these barriers, the humoral and cellular immune responses are activated (Strand and Pech, 1995; Ribeiro *et al*., 1999; Schmidt *et al*., 2001; Irving *et al*., 2005; Jiang *et al*., 2010; Tsakas and Marmaras, 2010). Humoral defenses in insects include some processes, such as the production of antimicrobial peptides (Meister *et al*., 2000; Lowenberger, 2001; Schmid-Hempel, 2005), reactive intermediates of oxygen or nitrogen (Bogdan *et al*., 2000; Vass and Nappi, 2001), and the complex enzymatic cascades that regulate coagulation or melanization of haemolymph (also called humoral encapsulation) (Muta and Iwanaga, 1996; Gillespie *et al*., 1997). The latter is consequent to the activation of the prophenoloxidase (proPO)-phenoloxidase (PO) system (Cerenius and Soderhall, 2004; Wang and Jiang, 2004; Xue *et al*., 2006; Cytrynska *et al*., 2007). Also, hemocytes play a role in the humoral defence by producing soluble effector molecules (Imler and Bulet, 2005; Kanost and Gorman, 2008).

In contrast, cellular immune defense refers to the immunocyte-mediated immune responses in insects, like phagocytosis, nodulation, encapsulation and clotting (Ribeiro and Brehelin, 2006; Tojo *et al*., 2000; Lavine and Strand, 2002; Strand, 2008; Browne *et al*., 2013; Vlisidou and Wood, 2015). It may be important to mention that the insect hemocytes perform necessary functions on the immune system, metabolism and detoxification, and also play an essential role in the defence of xenobiotics or microbial infection (Gupta, 1979). Together, circulating hemocytes and sessile hemocytes coordinate the insect

immunity against infection of pathogens (Hillyer and Strand, 2014; Hillyer, 2016). 'Apoptosis' is another immune defence in insects. It occurs generally against viral infections (Clem, 2005) and can be also induced by several environmental stimuli, such as UV-irradiation or chemicals (Kim *et al*., 2001). Furthermore, cell death caused by toxic agents has a different morphology and is called 'necrosis' (Wyllie, 1981). Because hemocytes are so sensitive against environmental impacts, the THC and apoptotic indices can be used as indicators for detecting the cytotoxic effects of chemicals (Altuntaş *et al*., 2012).

6.2. Insect Immunocytes:

The hemocytes of insects play a necessary role in defense against foreign bodies as these are the main immunocompetent cells of cellular immunity. In other words, hemocytes are generally accepted as cellular defense units responsible for the innate immune system in insects (Gupta *et al*., 2005; Ribeiro and Brehlin, 2006; Wood and Jacinto, 2007; Sharma *et al*., 2008). Depending on the stages of physiological development, the innate variability is observed among hemocytes within an insect species (Beetz *et al*., 2008). However, the type of immunocytes and their exact role in insects are still a debatable issue (Alfonso and Jones, 2002; Kanost *et al*., 2004).

As reported by many authors (Tojo *et al*., 2000; Ling and Yu, 2006; Kwon *et al*., 2014; Hwang *et al*., 2015), PLs and GRs are considered as key players in the cellular immune system, since their morphology is dramatically changed when they encounter the pathogens, as well as they have been observed engulfing and killing pathogens. For example, GRs are the predominant cell type in mosquitoes and play a key role in the cellular immune response (Castillo *et al*., 2006). In flies, PLs are the professional immune cell type and account for 95% of circulating hemocytes (Williams, 2007). In *D. melanogaster*, only PLs are involved in phagocytosis (Evans *et al*., 2003; Meister and Lagueux, 2003) while Crystal cells are non-phagocytic cells known to produce proPO, a necessary component of the melanization cascade (Binggeli *et al*., 2014).

Meanwhile, both types of hemocytes, PLs and GRs, play immune functions associated with phagocytosis and encapsulation in most Lepidoptera and some Coleoptera (Lavine and Strand, 2002; Manachini *et al*., 2011). Within Coleoptera, GRs are specialized to perform specific functions, such as phagocytosis and encapsulation in larvae of the beetle *Protaetia brevitarsis seulensis* (Kwon *et al*., 2014). In larvae of the same beetle, also, PLs occasionally engulfed bacteria and yeast (Hwang *et al*. (2015). In the beetle *Cetonischema aeruginos*, Giulianini *et al*. (2003) recorded phagocytosis of the latex beads *in vivo* by only GRs and OEs.

Among Lepidoptera, the immune response of caterpillars, including *G. mellonella*, *M. sexta*, *B. mori*, depends on the activities of two main hemocyte populations, GRs and PLs that recognize the pathogens and parasites (Nardi *et al*., 2003). These hemocyte types constitute approximately 90% of all hemocyte types in haemolymph and act as the key players in the immune system (Tojo *et al*., 2000; Nardi, 2004; Levin *et al*., 2005; İzzetoğlu and Karaçali, 2010). In addition, PLs of *M. sexta* larvae are the major hemocytes involved in phagocytosis of non-self-microsphere beads, whereas GRs are apparently the only hemocytes phagocytizing the self-dead cells (Ling and Yu, 2006). DHCs in *G. mellonella* larvae showed that PLs and GRs were the most abundant circulating cell types in the haemolymph (Wu *et al*., 2016).

6.3. Immuno-Suppressive Potential of Botanicals on Insects:

The activity of the immune system in insects depends on several factors, such as the population density (Reeson *et al*., 1998), food availability (Brown *et al*., 2009; Krams *et al*., 2014), and the environmental conditions, including temperature (Catalan *et al*., 2012). The relationships between temperature and the insect immune system function are close, probably because of the existence of cross-talk interactions between pathways

participating in responses of insects to temperature changes and immune stress (Sinclair *et al*., 2013). This possibility was substantiated by studies about *inter alia* participation of heat shock proteins in immune responses (Wojda *et al*., 2009; Zhu *et al*., 2013). However, the body of knowledge about the mechanisms and changes of the immune response to cold is relatively small. One of the few studies looking at this issue was that conducted by Urbanski *et al*. (2017). According to their results, the immune responses of the burying beetle *Nicrophorus vespilloides* were differently modulated by cold stress.

On the other hand, PLs and GRs in insects are known to give out cytoplasmic processes in retaliation to any invading foreign material and degeneration of their organelles (Gupta, 1985). Suppression of such processes of GRs in the *S. litura* larvae, after treatment with the essential oil of *Acorus calamus*, indicated the weakening of cellular defence reactions by this oil. The rapid degeneration of GRs, initiated by vacuolization and loss of firmness of organelles, leading to degranulation and a degenerative transformation within a period of 48 hr, further laid emphasis on the total collapse of the immunity-building mechanism of *S. litura* (Sharma *et al*., 2008).

Although many botanicals with the anti-mosquito property have been identified (Shaalan et *al*., 2005; Fallatah and Khater, 2010), very few of them have been tested for immunotoxicity in vector mosquitoes (James and Xu, 2012). Koodalingam *et al.* (2009, 2011) reported the effect of aqueous extract of the soap-nut Sapindus *emarginatus*, on the marker enzymes in the latter two developmental stages of Aedes *aegypti*. Then, Koodalingam *et al*. (2013) investigated the effect of aqueous extract of *S. emarginatus* on the 4th instar larvae and pupae of *A. aegypti* and recorded the immuno-suppressive potential or immunotoxicity of this plant extract in *A. aegypti*, for the first time. Also, Koodalingam *et al*. (2014) demonstrated, for the first time, that the NeemAzal differentially affected the immuno-suppressive state by reducing the phagocytic ability of hemocytes in larvae and pupae of *A. aegypti*.

6.4. Major Mechanisms of Cellular Innate Immune Responses in Insects:

6.4.1. *Phagocytosis:*

Specific insect haemocytes migrate towards and engulf several targets, including yeast, bacteria, apoptotic bodies, cell debris from damaged tissues and pathogens, in a process called 'phagocytosis' (Wood and Jacinto, 2007; Marmaras and Lampropoulou, 2009). According to the currently available literature, phagocytosis is generally classified into two categories: heterophagy and autophagy. Shortly, 'Heterophagy' is the phagocytic process for swallowing one cell by another to form intracellular phagosomes, which subsequently fuse with the endosomes and finally with the lysosomes leading to degradation of the foreign materials (Romao and Munz, 2011; Oczypok *et al*., 2013). In addition, 'Autophagy' is a process by which the cell degrades its own cytoplasmic content, such as protein aggregates and unnecessary organelles (Tettamanti *et al*., 2011; Kuballa *et al*., 2012) as a survival mechanism to maintain energy homeostasis (Colombo, 2007; Glick *et al*., 2010; Oczypok *et al*., 2013).

In insects, PLs and GRs are capable of phagocytizing abiotic particles, such as latex beads or India ink (Haszcz (2016). To study the phagocytic activity in linden bug *Pyrrhocoris apterus* adults and *S. littoralis* larvae, Berger and Jurčová (2012) conducted particle ingestion of the NBT test on PRs, GRs, PLs and SPs. According to their results, phagocytic activity was on average 10% in *P. apterus*, and 50% in *S. littoralis* haemocytes. However, some other hemocyte types may participate in phagocytosis and innate immune processes, as discussed before, in the present article. The effects of phytochemicals on phagocytosis in insects are scarcely reported in the available literature. Haszcz (2016) treated the *G. mellonella* larvae with essential oil of each *A. annua* and *Eucalyptus radiata*. These two oils did not change the hemocytic profile in treated larvae, i.e. they did not affect

the immune responses involving hemocyte mobilization or phagocytosis in larvae. On the contrary, *A. annua* extract influenced the phagocytic activity of hemocytes in *Eurygaster integriceps* (Zibaee and Bandani, 2010a). Because *A. annua* extracts suppressed the phagocytosis, at different concentration levels, these plant extracts 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 humoral components of the insect immune system are receptor-mediated (Ratcliffe and Rowley, 1987).

6.4.2. Encapsulation:

Encapsulation can be defined as a defence mechanism in which blood-borne foreign living (biotic) and non-living (abiotic) bodies are generally larger than those engulfed by phagocytosis (Strand, 2008). The cellular encapsulation results in a multilayer cellular capsule (overlapping layers of cells) surrounding the foreign bodies in insects (Götz and Boman, 1985). Also, encapsulation is known to begin within the first minutes after haemolymph penetration by a larger foreign body, where hemocytes attach to it forming a surrounding capsule (Marmaras and Lampropoulou, 2009; Dubovskii *et al*., 2010), Depending on the insect species and properties of the targeted object, capsules may be continually formed over 2-24 hr (Carton *et al*., 2008). In most cases, on the next day after the penetration by the invading body, the capsule is clearly visible but is considered fully complete only after 72 hr (Ratcliffe and Gagen, 1977; Dubovskiy *et al*., 2016).

In respect of the contribution of hemocytes in the encapsulation process, GRs were reported to contact a foreign targeted body, disintegrate or degranulate liberating material that endorses attachment of PLs and subsequently multiple layers of PLs from the capsule. According to the current literature, few studies have examined the effects of botanicals on encapsulation in insects. One of these few studies was conducted by Haszcz (2016). She implanted nylon monofilaments (one per larva) into *G. mellonella* larvae after treatment with neem essential oil. Her results showed that high doses of neem oil (1.5 mg/larva) significantly inhibited the degree of encapsulation in nylon implants. Therefore, she could conclude that neem essential oil impairs the immune system of *G. mellonella* larvae by inhibiting both PLs mobilization and encapsulation by hemocytes. In addition, Altuntaş *et al*. (2012) studied the impacts of different doses of GA_3 on the apoptosis, necrosis, encapsulation and melanization responses in *G. mellonella* larvae. Depending on their results, encapsulation rates of larval hemocytes were dependent on the extent of encapsulation and time but not treatment groups.

6.4.3. Nodulation

As reported by many authors (Lavine and Strand, 2002; Mullen and Goldsworthy, 2003; Marmaras and Lampropoulou, 2009), nodulation in insects is a cellular immune process whereby hemocytes recognize a foreign body and insulate it within the haemocoel as well as aggregate large numbers of invading bacteria. The enzyme PO in haemolymph can hydroxylate tyrosine and oxidize *o*-diphenols to quinones (Gorman *et al*., 2007). These quinones undergo a series of additional enzymatic and non-enzymatic reactions leading to melanin synthesis in the final stages of nodulation against invading material (Zibaee *et al*., 2011). Depending on the reported results of some authors (Zibaee *et al*., 2010, 2012), the nodulation and hemocyte spreading are suppressed in response to insecticides, insect growth regulators and plant products. To our knowledge, however, the available literature contains little information concerning the affected nodulation in insects by plant products. Azambuja *et al*. (1991) reported an inhibited nodulation response after Azt treatment to the last instar nymphs of *R. prolixus*. A year later, Azambuja and Garcia (1992) administrated Azt, *via* a blood meal, to the last instar nymphs of *R. prolixus* and recorded a reduction of immune reactivity, as indicated by a remarkable reduction in the nodule formation following challenge with the bacterium *Enterobacter cloacae* E 12. Also, Azt induced

permanent resistance to infection with the protozoan *Trypanosoma cruzi* in the vector of this parasite. In addition, Zibaee and Bandani (2010a) recorded an inhibitory effect of *A. annua* extract on the nodule formation in *E. integriceps*. After topical treatment of NeemAzal and injection of laminarin (a polysaccharide of glucose found in brown algae) into the last instar larvae of *G. mellonella*, Er *et al*. (2017) recorded a dose-dependent declination in the level of nodule formation.

6.5. Melanization As A Humoral Defense In Insects:

As previously highlighted, the complex enzymatic cascade that regulates coagulation or melanization of haemolymph (also called humoral encapsulation) is one of the humoral immune defenses in insects. As reported by Castillo *et al*. (2011), the melanization cascade overlaps the humoral and cellular defenses of the innate immune responses of insects. For some detail, PO (EC 1.14.18.1) activity plays an essential role in the innate immune responses of insects, which catalyzes the biosynthesis of quinones and other reactive intermediates to eliminate invading pathogens and parasites. Also, some authors (Cerenius *et al*., 2008; Kanost and Gorman, 2008) reported that PO plays a crucial role in melanin production during the cuticle sclerotization at external wound sites and during the defense responses, i.e., nodulation. However, melanization is initiated by cleavage of proPO to PO, an enzyme that can generate melanin upon oxidation of phenolic substrates (Christensen *et al*., 2005). During this reaction, toxic quinonoid substances, as well as other short-lived highly reactive oxygen and nitrogen intermediates are generated, and these are eventually involved in the formation of more long-lived stable products, such as melanin**,** known for its potent antimicrobial and antiparasitic properties (Hillyer *et al*., 2004; Cerenius *et al*., 2008). As reported by some authors (Ribeiro and Brehelin, 2006; Williams, 2007), the proPO is synthesized predominantly by hemocytes, especially in GRs and OEs.

With respect to the effects of plant extracts and products on the melanization of insect hemocytes *via* their effects on PO activity, application of essential oils of *O. sanctum, O. gratissimum* and *A. conyzoides* on *A. assama* resulted in a dose-dependent activation of PO activity and this might indicat ability of the essential oils to induce the immune response in larvae of the silkworm, *Antheraea assama* (Khanikor and Bora, 2012). Koodalingam *et al*. (2013) investigated the cuticular melanization responses upon injury in the 4th instar larvae of the mosquito *A. aegypti* after exposure to the kernel extract of *S. emarginatus*. They recorded an initial delay in the visible cuticular melanization reaction in the larvae, thereby indicating a possible impact of *S. emarginatus* kernel extract on the PO system of the *A. aegypti* larvae. Some years later, Huron *et al*. (2016) treated the adult females of the aphid *Aphis carricivora* with LC_{50} of acetone extracts of Lupine *Lupinus termis* and lemongrass *Cymbopogon citratus*. They recorded activation of POs, as well as the enzyme activity, was higher in aphids treated with *C. citratus* than that treated with *L. termis*. In addition, Sadeghi *et al*. (2017) treated the 4th instar larvae of *S. cretica* with 2500 ppm of the essential oil of *F. ovina* and recorded the highest PO activity 12 h post-treatment.

In contrast, Azt failed to interfere with the PO-activating systems in the late nymphal instars of the bug *R. prolixus*, since melanin synthesis or production was not reduced when this system is stimulated by tyrosin or by the presence of bacteria in the haemolymph (Azambuja *et al*., 1991). After the injection of Azt into the haemocoele of the locust *S. gregaria* by Annadurai and Rembold (1993), the production of immune proteins was induced. Also, Ayaad *et al*. (2001) injected Azt into haemocoele of the late larval instars of the flesh fly *P. surcoufi* and recorded an induction of the production of immune proteins and a drastic suppression of PO activity of haemolymph even when the activators laminarin, D-chymotrypsin and methanol were present. The impact of GA_3 on the

melanization response of *G. mellonella* larvae had been studied by Altuntaş *et al*. (2012**)**. Depending on their results, the extent of melanization of hemocytes exhibited a difference related to time, since inhibition of melanization was observed at 24 h-treated larvae, suggesting the negative impact of GA_3 on the cellular immune responses in these larvae.

6.6. Endocrine Control of Immune Defenses:

In the context of innate immune responses of insects to foreign materials, the endocrine regulation of the immune defense reactions should be highlighted. Although the relationship between endocrine organs and immune system had been reported in some insects (Gade *et al*., 1997; Rantala *et al*., 2003; Figueiredo *et al*., 2006; Franssens *et al*., 2006; Adamo, 2006; Beckage, 2008; Flatt *et al*., 2008), this issue is still disputable!! Figueiredo *et al*. (2006) proposed that ecdysone modulates the phagocytosis in *R. prolixus* nymphs. On the contrary, exposure of larvae of the corn earworm *Heliothis zea* and the tobacco budworm *Heliothis virescens* to ecdysone indicated that the encapsulation of their parasitoid *Cardiochiles nigriceps* eggs did not depend upon developmental or metamorphosis hormones (Lynn and Vinson, 1977). However, the process of encapsulation has been reduced after injection of JH into larvae of the mealworm beetle *Tenebrio molitor* (Sendi and Salehi, 2010). As reported by James and Xu (2012), the 20-hydroxyecdysone (20E) and juvenile hormone (JH) have shown two distinct effects on the cellular immunity of an insect since 20E induced the proliferation of hemocytes while JH (and its analogues) showed adverse results. On the other hand, the anti-JH compounds, precocious I and II, induced stronger encapsulation reactions in larvae of *S. littoralis* against its endoparasitoid *Microplitis rufiventris* (Khafagi and Hegazi, 2001). After treatment of last instar larvae of *G. mellonella* with Neemazal, or other botanicals, the decrease of THCs may be the reason for reduced nodulation and hemocyte spreading under the influence of neuroendocrine organs (Er *et al*., 2017).

7. Targeting the Insect Haemogram and Immune Reactions as A Pest Control Strategy:

The insect pests may be controlled by disturbing their physiological activities, *viz*. moulting, feeding, reproduction and immune system (Pandey *et al*., 2012**)**. It is important to mention that the insect hemocytes perform important functions in metabolism and detoxification, and also play an essential role in the defence of xenobiotics or the immunity against microbial infection (Hillyer and Strand, 2014; Hillyer, 2016). The insect haemogram profile, i.e., THC, DHCs, BV, MI, cytopathological features of hemocytes and heart activity, represents a very good indicator of the insect physiology and the environmental adaptability in each stage of the insect development (Giglio *et al*., 2008; Sharma *et al*., 2008; Ghasemi *et al*., 2013a, b; Bardoloi *et al*., 2016). Also, the insect haemogram is suggested to be a valuable tool for the investigation of the toxic effects of the insecticidal materials on biocontrol agents (Kohlmaier and Edger, 2008; Qamar and Jamal, 2009). In this section, we would like to shed some light on searching for a new strategy for pest control by plant extracts and products through their disruptive effects on the haematological parameters and immune reactions of insect pests.

As previously shown, different plant extracts and products are found to exhibit impairing effects on different parameters of the insect haemogram. For example, the altered THC and the cytopathological features of hemocytes in insects are frequently used to demonstrate the cytogenetic damage caused by botanicals (Yeh *et al*., 2005; Altuntaş *et al*., 2012; Pandey *et al*., 2012). The results of some studies revealed that different botanicals cause much variation in the proportions of hemocyte populations in various insects (Er *et al*., 2017; Shaurub and Sabbour, 2017) In addition, the cytopathological effects of plant extracts and products on the hemocytes have been reported in a few insect species (Sharma

et al., 2001, 2003, 2008). Also, the antimitotic effects of some botanicals have been demonstrated in the cell lines of various insect species (Salehzadeh *et al*., 2003; Huang *et al*., 2011; Er and Keskin, 2016; Çelik *et al*., 2017). Moreover, the influence of botanicals on insect heart activity was recorded in few studies (Marciniak *et al*., 2010; Ntalli *et al*., 2014).

In respect of the immune reactions in insects and their disruption by some plant extracts and products, many authors (Giglio *et al*., 2008; Sharma *et al*., 2008; Ghasemi *et al*., 2013a, b; Bardoloi *et al*., 2016) reported that the adverse effects of botanicals on various haemogram parameters reflect the suppression of immune capability in insects. For example, THC in haemolymph reflects the immune ability for dealing with pathogens or foreign chemicals (Asiri, 2017) since the reduction in THC after treatment with certain botanicals may be attributed to the formation of nodules, encapsulation and apoptosis (Sharma *et al*., 2003; Pandey *et al*., 2007) and also to the inhibition of larval hematopoietic function and cell proliferation (Zhu *et al*., 2012). In addition, the reduction of THC in botanical-treated insects might have resulted from their toxic effects on the immunocytes (Sadeghi *et al*., 2017**)**. Therefore, the plant extracts and plant-derived materials suppress the immune capability, leading to the insects become susceptible to the effects of pathogenic microorganisms (Zibaee *et al*., 2012) and ultimately death. By compromising the insect's innate immunity, one can develop a new strategy for effective control of insect pests (for some reviews, see James and Xu, 2012; Haszcz, 2016; Liu *et al*., 2017).

8. Summary points

In the current article, the majority of discussed aspects can be summarized in the following points.

* The indiscriminate and excessive uses of conventional insecticides lead to several drastic problems in the environment, human health and economics. Therefore, it is necessary to search for safe alternative materials among which botanicals represent an effective alternative for pest control *via* influencing the haemogram parameters and immune reactions.
* Haemogram is a quantitative (Total haemocyte count, THC) and qualitative (Differential haemocyte count, DHC) expression of the haemolymph and its constituent inclusions. Haemogram parameters include, also, haemolymph (blood) volume, mitotic index and cytological features of hemocytes.
* THC in different insects increases in response to the botanical treatment. A number of scenarios had been provided to interpret these THC increases. On the contrary, decreasing THC had been reported in several insects by various plant extracts or plant-derived substances. Also, different reasons for THC decreasing were suggested.
* In many insects, DHCs are altered as a response to various plant extracts or phytochemicals. Each type of circulating hemocytes increased or decreased in haemolymph depending on the tested plant, solvent and concentration level as well as on the susceptibility of the insect developmental stage and age. However, some conceivable interpretations had been suggested for the alteration in a population of each of the major hemocyte types.
* The histopathological effects of plant extracts and botanical products on different hemocyte types have been reported in few insect species, including the extrusion of nuclear material, pycnosis, karyorrhexis, denucleation, abnormal changes in shape, volume, distribution, or configuration of hemocytes, cytoplasmic vacuolation, protrusions and/or projections, cell lysis, bulging of cytoplasm, etc. Also, the histopathological effects of botanicals on insect hemocytes had been discussed.
* There are other parameters of affected haemogram by botanicals, such as the blood

volume, mitotic indices but very few studies focused on the effects of plant extracts and phytochemicals on the heartbeat rate and heart contractile activity of insect species.
* Insects appear to lack the acquired immune response characteristic of vertebrates. Therefore, insects rely solely on their highly efficient innate immunity for defense against pathogens and other invaders. The innate immune system of insects involves cellular and humoral defence mechanisms.
* The plasmatocytes (PLs) and Granulocytes (GRs) can be considered as key players in the cellular immune system in insects. The weakening of the cellular defence reactions had been recorded in some insects by certain plant materials.
* Phagocytosis is a cellular innate immune mechanism in insects. The phagocytic ability of hemocytes PLs and GRs (and maybe other hemocytes) of larvae and pupae of some insects had been suppressed as a response to the botanical treatments. Nevertheless, some botanicals did not affect the immune responses.
* Encapsulation is a cellular innate immune mechanism in insects. Encapsulation is a defence mechanism in which blood-borne foreign living (biotic) and non-living (abiotic) bodies are generally larger than those engulfed by phagocytosis. Multiple layers of PLs from the capsule containing a foreign target. Few studies have examined the effects of plant extracts on encapsulation in insects.
* Nodulation (Nodule formation) is a cellular innate immune mechanism in insects. To our knowledge, however, little information is available concerning the affected nodulation process in insects by plant products. Inhibited nodulation responses in larvae of some insects were observed after treatment with Azadirachtin, other neem formulations, or other plant extracts, following a challenge with some bacteria.
* With regard to the humoral innate immunity in insects, Phenoloxidase (PO) activity plays a key role in the innate immune responses, because it catalyzes the biosynthesis of quinones and other reactive intermediates to eliminate the invading pathogens and parasites. Melanization is initiated by a cleavage of prophenoloxidase (synthesized predominantly by hemocytes, especially in GRs and OEs) to PO, for generating melanin. The effects of some botanicals on the melanization of insect hemocytes can be explained by their effects on PO activity.
* The neuroendocrine organs, such as those producing developmental hormones, have been reported to affect the immune responses in insects. The decrease of THC in the larvae after treatment with some botanicals may be due to the reduced nodulation and hemocyte spreading under the control of some developmental hormones.
* The influenced THC and/or DHCs in many insects after treatment with some botanicals may be attributed to the phagocytosis, nodule formation, encapsulation and apoptosis and also to the inhibition of larval hematopoietic function. Therefore, the botanicals suppress the immune capability, leading to the insects become susceptible to the effects of microbes and ultimately results in death. This can be appreciated as a new strategy for an effective control approach of insect pests.

9. Conclusions and Future Research Needs:

As clearly shown in the present article, different plant extracts and products are found to exhibit various impairing effects on different parameters of the insect haemogram, such as alteration of THC and DHCs, as well as cause detrimentally structural abnormalities of the hemocytes and reduction of hemocyte mitosis. Also, the influence of phytochemicals on insect heart activity was reported. The disturbed THC and/or DHCs in insects after treatment with botanicals may be attributed to the formation of nodules, encapsulation and apoptosis and also to the inhibition of larval hematopoietic function. Therefore, the botanicals suppress the immune capability, leading to the insects become

susceptible to the effects of microbes and ultimately death. This can be considered as a new strategy for an effective control approach of insect pests. In this context, some points of research need more investigation in the future, such as isolation of the active ingredients in plant extracts, antimitotic activities of botanicals and their adverse effects on the heartbeat rate, blood volume in insects, as well as the hormonal properties of botanicals influencing the haemogram variables and immune responses in insects. In addition, some fieldwork should be conducted to realize the botanical potential for the recommendation of this approach of pest control.

REFERENCES

Abhilash, P.C. & Singh, N. (2009). Pesticide use and application: An Indian scenario. *Journal of Hazardous Materials,* 165(1): 1-12.DOI:10.1016/j.jhazmat.2008.10.061

Adamo, S.A. (2006). Comparative psychoneuroimmunology: Evidence from the insects. *Behavioral Cognitive Neuroscience Review,* 5(3), 128-140. https://doi. org/ 10. 1177/1534582306289580

Adline, J.D., Gunasekaran, J.C. & Lingathurai, S. (2015). Toxic effect of *Glinus lotoides* (Aizoaceae) on rice-moth, *Corcyra cephalonica* Staintion (Lepidoptera: Pyralidae) eggs. *International Journal of Current Research in Biosciences and Plant Biology,* 2(2), 35-42.

Ajamhassani, M., Sendi, J.J., Zibaee, A., Askary, H. & Farsi, M.J. (2013). Immunoliogical responses of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) to entomopathogenic fungi, *Beauveria bassiana* (Bals. -Criy) and *Isaria farinosae* (Holmsk.). *Journal of Plant Protection Research,* 53(2), 110-118. DOI: https:// doi.org/10.2478/jppr-2013-0016

AlFonso T.B. & Jones, B.W. (2002). Gcm2 promotes glial cells differentiation and is required with glial cells missing for macrophage development in *Drosophila*. *Developmental Biology*, 248, 369-383. doi:10.1006/dbio.2002.0740

Al-Ghamdi, M.S. (2001). The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. *Journal of Ethnopharmacology,* 76(1), 45-48. https://doi.org/ 10. 1016/S0378-8741(01)00216-1

Ali, B.H. & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research,* 17(4), 299-305. https://doi.org/10.1002/ptr.1309

Ali, M.A., Sayeed, M.A., Alam, M.S., Yeasmin, M.S., Khan, A.M. & Muhamad, I.I. (2012). Characteristics of oils and nutrient contents of *Nigella sativa* Linn. and *Trigonella foenum-graecum* seeds. *Bulletin of Chemical Society Ethiopia*, 26, 55–64.

Altuntaş, H., Kiliç, A.Y., Uçkan, F. & Ergin, E. (2012). Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Environmental Entomology,* 41(3), 688-696. https://doi.org/10.1603/EN11307

Anderson, R.S., Burreson, E.M. & Paynter, K.T. (1995). Defense responses of haemocytes withdrawn from *Crassostrea virginica* infected with *Perkinsus marinus*. *Journal of Invertebrate. Pathology,* 66, 82-89. https://doi.org/10.1006/jipa.1995.1065

Annuradha, I.R.S. & Rembold, H. (1993). Azadirachtin A modulates the tissue specific 2D polypeptide pattern of the desert locust, *Schistocerca gregaria*. *Naturwissenschaften,* 80, 127–130.

Annuradha, A. & Anuadurai, R.S. **(**2008). Biochemical and molecular evidence of azadirachtin binding to insect actions. *Current Science,* 95(11), 1588-1593. https://www.jstor.org/stable/24105517

Arnold, J.W. (1952). Effects of certain fumigants on haemocytes of the Mediterranean flour

moth, *Ephestia kuhniella* Zell. (Lepidoptera, Pyralididae). *Canadian Journal of Zoology*, 30, 365-374. https://doi.org/10.1139/z52-032

Arnold, J.W. (1972). A comparative study of the haemocytes (blood cells) of cockroaches (Insecta, Dictyoptera, Blattari), with a view of their significance in taxonomy. *The Canadian Entomologist*, 104, 309-348. DOI: 10.4039/Ent104309-3

Arvy, L., Gabe, M. & Lhoste, J. (1950). Action de quelques insecticides sur le sang du Doryphore (*Chrysomela decemlineata* Say.). *Bulletin de la Société entomologique de **France***,55,122-127.

Ashida, M. & Brey, P.T. (1995). Role of the integument in insect defense: prophenoloxidase cascade in the cuticular matrix. *Proceeding of the National Academy of Sciences, U S A*, 92, 10698–10702. https:// doi.org/ 10.1073/ pnas. 92. 23.10698

Asiri, B.M.K. (2017). Bioinsecticides induce change in biochemical and immunological parameters of *Spodoptera littoralis* larvae. *Chemical and Biomolecular Engineering*, 2(2), 106-112. doi: 10.11648/ j.cbe. 20170202.15

Ayaad, T.H., Dorrah, M.A., Shaurub, E.H. & El-Sadawy, H.A. (2001). Effects of the entomopathogenic nematode, *Heterohabditis bacteriophora* HP88 and azadirachtin on the immune defense response and prophenoloxidase of *Parasarcophaga surcoufi* larvae (Diptera: Sarcophagidae). *Journal of Egyptian Society of Parasitology*, 31(1), 295-325.

Aydin, H. & Gurkan, M.O. (2006). The efficacy of spinosad on different strains of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Turkish Journal of biology*, 30, 5-9.

Azambuja, P.D. & Garcia, E.S. (1992). Effects of azadirachtin on *Rhodnius prolixus*: immunity and *Trypanosoma* infection. *The Memórias **do Instituto Oswaldo Cruz***, 87 (Suppl. 5), 69–72.

Azambuja, P.D., Garcia, E.S., Ratcliffe, N.A. & Warthen, J.D. (1991). Immune-depression in *Rhodnius prolixus* induced by the growth inhibitor azadirachtin. *Journal of Insect Physiology*, 37, 771–777. https://doi. org/ 10.1016/0022-1910(91)90112-D

Baishya, B.P., Bardoloi, S. & Bharali, R. (2015). Ultrastructure of the hemocytes of Muga silkworm larva *Antheraea assama* Westwood (Lepidoptera; Saturniidae): a phase-contrast and electron microscopic study. *International Journal of Pure and Applied Biosciences*, 3, 234-240.

Baishya, B.P., Bardoloi, S. & Bharali, R. (2016). Morphological changes in the hemocytes of *Antheraea assama* (Lepidoptera: Saturniidae) upon bacterial infection. *Journal of Entomology and Zoology Studies*, 4(6), 46-49.

Bardoloi, S., Desdimona, K. & Mazid, S. (2016). Comparative study of the changes in haemogram of *Antheraea assama* Ww reared on two host plants, Som (*Machilus bombycina* King) and Soalu (*Litsea polyantha* Juss). *International Journal of Pure and Applied Bioscience*, 4(5), 144-152. DOI: 10.18782/2320-7051.2368

Barnby M.A. & J.A. Klocke, (1990). Effects of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Fabr) larvae. *Journal of Insect Physiology*, 36, 125-131. doi:10.1016/0022-1910(90)90183-G

Beaulaton, J. (1979). Haemocytes and haemocytopoiesis in silkworms. *Biochimie*, 61, 157-164.

Beckage, N.E. (2008). Insect Immunology. Academic press. 348 pp.

Beetz, S., Holthusen, T.K., Koolman, J. & Trenczek, T. (2008). Correlation of hemocyte counts with different developmental parameters during the last larval instar of the tobacco hornworm, *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, 67(2), 63-75. DOI: 10.1002/arch.20221

Ben Hamouda, A., Mechi, A., Zarrad Kh., Laarif A. & Chaieb, I. (2015). Disruptive effects of pomegranate *Punica granatum* Linn. (Lythraceae) extracts on the feeding, digestion and morphology of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Entomology and Applied Science Letters*, 2(2), 1-6.

Berger, J. & Jurčová, M. (2012). Phagocytosis of insect haemocytes as a new alternative model. *Journal of Applied Biomedicine*, 10, 35-40. https://doi. org/ 10.2478/ v10136-012-0003-1

Bergin, D., Reeves, E.P., Renwick, J., Wientjes, F.B., Kavanagh, K. (2005). Superoxide production in *Galleria mellonella* hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. *Infection and Immunity*, 73, 4161-4170.

Binggeli, O., Neyen, C., Poidevin, M. & Lemaitre, B. (2014). Prophenoloxidase activation is required for survival to microbial infections in *Drosophila*. *PLOS Pathogens*,10: e1004067.doi: 10.1371/ journal. ppat.1004067.

Blanco, L.A.A. (2016). Differential cellular immune response of hemocyte of *Galleria mellonella* larvae against *Actinobacillus pleuropneumoniae* Strains. M.Sc. Thesis, Universidade Federal de Viçosa, Brazil.

Bogdan, C., Röllinghoff, M. & Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion Immunology*, 12. 64–76. https://doi.org/10.1016/S0952-7915 (99) 00052-7

Bokaeian, M., Saboori, E., Saeidi, S., Niazi, A.A., Amini-Borojeni, N., Khaje, H. & Bazi, S. (2013). Phytochemical Analysis, Antibacterial Activity of Marrubium *vulgare* L. against Staphylococcus *aureus in vitro*. *Zahedan Journal of Research in Medical Sciences*, 16(10), 60-64.

Brayner, F.A., Araújo, H.R., Santos, S.S., Cavalcanti, M.G., Alves, L.C., Souza, J.R. & Peixoto, C.A. (2007). Hemocyte population and ultrastructural changes during the immune response of *Culex quinquefasciatus* (Diptera: Culicidae) to microfilariae of *Wuchereria bancrofti*. *Medical and Veterinary Entomology*, 21, 112-120. DOI: 10.1111/j.1365-2915. 2007.00673.x

Brehelin, M. & Hoffman, J.A. (1980). Phagocytosis of inert particles in *Locusta migratoria* and *Galleria mellonella*: study of ultrastructure and clearance. *Journal of Insect Physiology*,26, 103-111.doi:10.1016/0022-1910 (80)90049-9

Brown, A.E., Baumbach, J., Cook, P.E. & Ligoxygakis, P. (2009). Short-term starvation of immune deficient *Drosophila* improves survival to gram-negative bacterial infections. *PLoSONE*, 4: e4490. https://doi.org/10.1371/ journal. pone.0004490

Browne, N., Heelan, M. & Kavanagh, K. (2013). An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes. *Virulence*, 4, 597–603. http://dx.doi. org/10. 4161/viru.25906

Butt, T.M. & Shields, K.S. (1996). The structure and behaviour of Gypsy moth (*Lymantria dispar*) hemocytes. *Journal of Invertebrate Pathology*, 68, 1-14. https://doi.org/10.1006/jipa.1996.0052

CAPE (2009). Position statement on synthetic pesticides. http://www.cape.ca/ toxicspesticidesps.html.

Carton Y., Poirie M. & Nappi AJ. (2008). Insect immune resistance to parasitoids. *Insect Science*, 15, 67-87. https://doi.org/10.1111/j.1744-7917.2008.00188.x

Castillo, J.C., Robertson, A.E. & Strand, M.R. (2006). Characterization of hemocytes from the mosquitoes *Anophles gambiase* and *Aedes aegypti*. *insect biochemistry and insect molecular biology*, 36, 891-903.

Castillo, J.C., Reynolds, S.E. & Eleftherianos, I. (2011). Insect immune responses to nematode parasites. *Trends in Parasitology*, 27, 537-547. https: //doi.org/10.1016/

j.pt.2011.09.001

Catalan, T.P., Wozniak, A., Niemeyer, H.M., Kalergis, A.M. & Bozinovic, F. (2012). Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *Journal of Insect Physiology,* 58, 310-317. https://doi.org/10. 1016/ j.jinsphys. 2011. 10.001

Çelik, D., Özbek, R. & Uckan, F. (2017). Effects of indole-3-acetic acid on hemocytes of *Achoria grisella* Fabr. (Lepidoptera: Pyralidae). *Journal of Entomological Research Society,* 19(2), 83-93.

Ceraul, S.M., Sonenshine, D.E., Ratzlaff, R.E. & Hynes, W.L. (2003). An arthropod defensin expressed by the hemocytes of the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). *insect biochemistry and insect molecular biology*, 33, 1099-1103.

Cerenius, L. & Soderhall, K. (2004). The prophenoloxidase-activating system in invertebrates. Immunol. Rev., 198, 116–126. https://doi.org/ 10.1111/j.0105-2896. 2004.00116.x

Cerenius, L., Lee, B.L. & Söderhäll, K. (2008). The proPO system: pros and cons for its role in invertebrate immunity. *Trends in Immunology*, 29, 263–271. DOI:10. 1016/ j.it.2008.02.009

Chapman, R.F. (1982). The Insect Structure and Function. E.L.B.S. Edition, pp: 92-94.

Charalambidis, D.N., Foukas, C.L., Zervas, G.C. & Marmaras, J.V. (1996). Haemocyte surface phenoloxidase (PO) and immune response to lipopolysaccharide (LPS) in *Ceratitis capitata*. *insect biochemistry and insect molecular biology*, 26, 867-874. https://doi.org/10.1016/S0965-1748(96)00053-7

Charroux, B. & Royet, J. (2009). Elimination of plasmatocytes by targeted apoptosis reveals their role in multiple aspects of the *Drosophila* immune response. *Proceedings of National Academy Sciences, U.S.A.,* 106, 9797–9802. https://doi. org/10.1073/ pnas. 0903971106

Chavan, J.A., Chougale, A.K. & Bhawane, G.P. (2017). Toxicity of Dimethoate and Chlorpyriphos on haemocyte count in male *Platynotus belli* Fairmaire (Coleoptera: Tenebrionidae). *Journal of Entomology and Zoology Studies,* 5(1), 126-133.

Chennaiyan, V., Sivakami, R. & Jeyasankar, A. (2016a). Effect of *Duranta erecta* Linn (Verbenaceae) leaf extracts against armyworm *Spodoptera litura* and cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *International Journal of Advanced Research of Biological Scienes,* 3(2), 311-320.

Chennaiyan, V., Sivakami, R. & Jeyasankar, A. (2016b). Evaluating ecofriendly botanicals of *Barleria longiflora* Linn. F. (Acanthaceae) against armyworm *Spodoptera litura* Fab. and cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Annual Research and Review of Biology,* 10(3), 1-9.

Christensen, B.M., Li, J., Chen, C.C. & Nappi, A.J. (2005). Melanization immune responses in mosquito vectors. *Trends in Parasitology,* 21, 192–199. http://sciencedomain.org/review-history/14627

Chu, F.L.E., La-Peyre, J.F. & Burreson, C.S. (1993). *Perkinsus marinus* infection and potential defense-related activities in *Eastern oysters, Crassostrea virginica*: Salinity effect. *Journal of Invertebrate. Pathology,* 62, 226- 232. https://doi.org/ 10. 1006/ jipa. 1993.1104

Clem, R.J. (2005). The role of apoptosis in defense against baculovirus infection in insects. *Current Topics in Microbiology and Immunology,* 289, 113-129. https://doi.org/10. 1007/3-540-27320-4_5

Clemente, S., Mareggiani, G., Broussalis, A., Martino, V. & Ferraro, G. (2003). Insecticidal

effects of Lamiaceae species against stored products insects. *Boletín de Sanidad Vegetal. Plagas*, 29, 1-8.

Colombo, M.I. (2007). Autophagy: a pathogen driven process. *Life*, 59, 238-242. DOI: 10.1080/15216540701230503

Costa, S.C.P., Ribeiro, C., Girard, P.A., Zumbihl, R. & Brehelin, M. (2005). Modes of phagocytosis of Gram-positive and Gram-negative bacteria by *Spodoptera littoralis* granular haemocytes. *Journal of Insect Physiology,* 51, 39-46. https://doi.org/ 10. 1016/j.jinsphys. 2004. 10.014

Costa, L.G., Giordano, G., Guizzetti, M. & Vitalone, A. (2008). Neurotoxicity of pesticides: a brief review. *Frontiers BioScience,* 13, 1240-1249. DOI: 10. 2741/ 2758

Cytrynska, M., Mak, P., Zdybicka-Barabas, A., Suder, P. & Jakubowicz, T. (2007). Purification and characterization of eight peptides from *Gellaria mellonella* immune hemolymph. *Peptides,* 28, 533–546. https://doi.org/10.1016/j .peptides. 2006.11.010

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. & Williamson, M.S. (2007). DDT, pyrethrins and insect sodium channels. *IUBMB Life,* 59, 151-162. DOI: 10.1080/ 15216540701352042

Dayan, F.E., Cantrell, C.L. & Duke, S.O. (2009). Natural products in crop protection. *Bioorganic and Medical Chemistry letters,* 17(12), 4022-4034. https://doi.org/ 10. 1016/j.bmc. 2009. 01.046

de Silva, C., Dunphy, G.B. & Rau, M.E. (2000). Interaction of hemocytes and prophenoloxidase system of fifth instar nymphs of *Acheta domesticus* with bacteria. *Developmental and Comparative Immunology,* 24, 367-379. https://doi.org/10. 1016/S0145-305X(99)00063-4

Derbalah, A.S., Khidr, A.A., Moustafa, H.Z. & Taman, A. (2014). Laboratory evaluation of some non-conventional pest control agents against the pink bollworm *Pectinophora gossypiella* (Saunders). *Egyptian Journal of Biological Pest Control,* 24(2), 363-368. http://www.esbcp.org/index.asp

Dubey, N.K., Shukla, R., Kumar, A., Singh, P. & Prakash, B. (2010). Prospects of botanical pesticides in sustainable agriculture. *Current Science*, 98(4), 479-480.

Dubey N.K., Shukla R., Kumar A., Singh P. & Prakash B. (2011). Global scenario on the application of natural products in integrated pest management programmes. In: "Natural Products in Plant Pest Management"(Dubey, N.K., ed.), chapter1, pp: 1-20.

Dubovskii, I.M., Grizanova, E.V., Chertkova, E.A., Slepneva, I.A., Komarov, D.A., Vorontsova, Y.L. & Glupov, V. (2010). Generation of reactive oxygen species and activity of antioxidants in hemolymph of the moth larvae *Galleria mellonella* (L.) (Lepidoptera: Piralidae) at development of the process of encapsulation. *Journal of Evolutionaty Biochemistry and Physiology,* 46, 35-43. https://doi.org/10.1134/ S0022093010010044

Dubovskiy, I.M., Kryukova, N.A., Glupov, V.V. & Ratcliffe, N.A. (2016). Encapsulation and nodulation in insects. *Invertebrate Survival Journal,* 13, 229-246.

Dunphy, G.B. & Nolan, R.A. (1980). Hemograms of selected stages of the spruce budworm, *Christoneura fumiferana* (Lepidoptera: Tortricidae). *The Canadian Entomologist,* 112(5), 443-450. DOI: 10.4039/Ent112443-5

El-Sheikh, T.A.A. (2002). Effects of application of selected insect growth regulators and plant extracts on some physiological aspects of the black cutworm, *Agrotis ipsilon* (HUF.). Ph.D. Thesis, Faculty of Science, Ain Shams University, Egypt.

Er, A. & Keskin, M. (2016). Influence of abscisic acid on the biology and hemocytes of

the model insect *Galleria mellonella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America,* 109(2), 244-251. https://doi.org/10.1093/ aesa/ sav122

Er, A., Uçkan, F., Rivers, D.B., Ergin, E. & Sak, O. (2010). Effects of parasitization and envenomation by the endoparasitic wasp *Pimpla turionellae* (Hymenoptera: Ichneumonidae) on hemocyte numbers, morphology, and viability of its host *Galleria mellonella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America,* 103, 273-82. https://doi.org/10.1603/AN09065

Er, A., Taşkıran, D. & Sak, O. (2017). Azadirachtin-induced effects on various life history traits and cellular immune reactions of *Galleria mellonella* (Lepidoptera: Pyralidae). *Archives of Biological Science,* 69(2), 335-344. https://doi.org/10.2298/ ABS160421108E

Essawy, M., Maleville, A. & Brehelin, M. (1985). The haemocytes of *Heliothis armigera*: ultrastructure, cytochemistry and functions. *Journal of Morphology,* 186, 255–264. https://doi.org/ 10.1002/ jmor. 1051860302

Evans, C.J., Hartenstein, V. & Banerjee, U. (2003). Thicker than blood: conserved mechanisms in *Drosophila* and vertebrate hematopoiesis. *Developmental Cell,* 5, 673–690. doi: 10.1016/S1534-5807(03)00335-6.

Fallatah, S.A. & Khater, E.I. (2010). Potential medicinal plants in mosquito control. *Journal of Egyptian Society of Parasitology*, 40, 1–26.

Farag, N.A. (2002). Effect of *Haphyllum tuberculatum* extracts on an insect on an insect pest *Spodoptera littoralis* and the predator, *Coccinella undecimpunctata*. *Annals of Agricultural Sciences, Cairo,* 47, 1097-1105.

Feitosa, A.P., Alves, L.C., Chaves, M.M., Veras, D.L., Silva, E.M., Aliança, A.S., França, I.R., Gonçalves, G.G., Lima-Filho, J.L. & Brayner, F.A. (2015). Hemocytes of *Rhipicephalus sanguineus* (Acari: Ixodidae): characterization, population abundance, and ultrastructural changes following challenge with *Leishmania infantum*. *Journal of Medical Entomology,* 52, 1193-202. https://doi.org/ 10.1093/ jme/ tjv125

Feliciano, D.F., Bassani, R.A., Oliveira, P.X. & Bassani, J.W. (2011). Pacemaker activity in the insect (*T. molitor*) heart: role of the sarcoplasmic reticulum. *American Journal of Physiology, Regulatory Integrative and Comparative Physiology,* 301(6), 1838-1845. https://doi.org/10. 1152/ajpregu.00089.2011

Figueiredo, M.B., Castro, D.P., Nogueira, N.F.S., Garcia, E.S. & Azambuja, P. (2006). Cellular immune response in *Rhodnius prolixus*: role of ecdysone in haemocyte phagocytosis. *Journal of Insect Physiology,* 52, 711-716. https://doi.org/10. 1016/ j. jinsphys.2006.03.011

Flatt, T., Heyland, A., Rus, F., Porpiglia, E., Sherlock, C., Yamamoto, R., Garbuzov, A., Palli, S.R., Tatar, M. & Silverman, N. (2008). Hormonal regulation of the humoral innate immune response in *Drosophila melanogaster*. *The Journal of Experimental Biology,* 211(16), 2712-2724. doi: 10.1242/jeb.014878

Franssens, V., Smagghe, G., Simonet, G., Claeys, I., Breugelmans, B., DeLoof, A. & Vanden, B.J. (2006). 20-Hydroxyecdysone and juvenile hormone regulate the laminarin-induced nodulation reaction in larvae of the flesh fly, *Neobellieria bullata*. *Developmental and Comparative Immunology,* 30(9), 735–740. https://doi.org/10.1016/j. dci.2005.10.010

Gad, A.A. & El-DaKheel, A.A. (2009). Larvicidal activities of *Cinnamomum osmophloeum* and *Matricharia chamomella* extracts against the filarial mosquito *Culex quinquefasciatus* (Diptera: Culicidae) and their effects on its haemogram. *The Egyptian Science Magazine,* 6(1/2), 8-15.

Gade, G., Hoffman, K.H. & Spring, J.H. (1997). Hormonal regulation in insets: facts, gaps, and future directions. *Physiological Reviews,* 77(4), 963-1032. DOI: 10.1152/ physrev.1997.77.4.963

Gandhe, A.S., John, S.H. & Nagaraju, J. (2007). Noduler, A novel immune up-regulated protein mediates nodulation response in insects. *Journal of Immunology,* 179, 6943-6951. https://doi.org/ 10.4049/ jimmunol. 179. 10. 6943

Garcia, G.E. & Rosales, C. (2002). Signal transduction during Fc receptor-mediated phagocytosis. *Journal of Leukocyte Biology,* 72, 1092-1108. https://doi.org/10.1189/jlb.72.6. 1092

Gardiner, E.M.M. & Strand, M.R. (2000). Hematopoiesis in larval *Pseudoplusia includes* and *Spodoptera frugiperda*. *Archives of Insect Biochemistry and Physiology,* 43, 147–164.DOI:10.1002/(SICI)1520-6327(200004)43:4<147::AID-ARCH1>3.0. CO;2-J

George, P.J.E. (1996). Impact of chosen insecticides on three non-target reduviid biocontrol agents (Insecta: Heteroptera: Reduviilidae). Ph.D. Thesis, Triunelveli: Manonmaniam Sundaranar University, Tamil Nadu, India. 117pp.

George, P.J.E. & Ambrose, D.P. (2004). Impact of insecticides on the haemogram of *Rhynocoris kumarii* Ambrose & Livingstone (Hem. Reduviidae). *Journal of Applied Entomology,* 128(9-10), 600-604. https://doi.org/10.1111/j.1439-0418. 2004. 00896.x

Ghasemi, V., Moharramipour, S. & Sendi, J.J. (2013a). Circulating hemocytes of Mediterranean flour moth, *Ephestia kuehniella* Zell. (Lep: Pyralidae) and their response to thermal stress. *Invertebrate Survival Journal,* 10, 128-140.

Ghasemi, V., Yazdib, A.K., Tavallaie, F.Z. & Sendi, J.J. (2013b). Effect of essential oils from *Callistemon viminalis* and *Ferula gummosa* on toxicity and on the hemocyte profile of *Ephestia kuehniella* (Lep.: Pyralidae). *Archives of Phytopathology and Plant Protection,* 47(3), 268-278. https://doi.org/10.1080/ 03235408.2013.808856

Ghoneim, K. (2019). Characterization of qualitative and quantitative haemogram parameters in insects: a review of current concepts and future prospects. *Egyptian Academic Journal of Biological Sciences (A. Entomology),* 12(1): 9-63.

Ghoneim, K., Hamadah, Kh., El-Hela, A., Mohammad, A. & Amer, M. (2015a). Efficacy of *Nigella sativa* (Ranunculaceae) extracts on adult performance and phase transition of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). *International Journal of Research Studies in Zoology,* 1(2), 29-44.

Ghoneim, K., Hamadah, Kh., El-Hela, A. & Amer, M. (2015b). Inhibitory effects of *Nigella sativa* (Ranunculaceae) extracts on the reproduction of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). *International Journal of Research Studies in Zoology,* 1(1), 43-55.

Ghoneim, K., Tanani, M., Hamadah, Kh., Basiouny, A. & Waheeb, H. (2015c). Effects of Novaluron and Cyromazine, chitin synthesis inhibitors, on the larval haemogram of *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). *International Journal of Advanced Research,* 3(1), 554 -576.

Ghoneim, K., Hamadah, Kh., Amer, M., El-Hela, A. & Mohammad, A. (2015d). Qualitative and quantitative changes in the haemogram of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) by extracts of *Nigella sativa* (Ranunculaceae). *Journal of Advances in Biology,* 7(2), 1275-1292.

Ghoneim, K., Hassan, H.A., Tanani, M.A. & Bakr, N.A. (2017). Toxic and disruptive effects of Novaluron, a chitin synthesis inhibitor, on development of the pink bollworm *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelechiidae). *International Journal of Entomology Research,* 2(2), 36-47.

Giglio, A., Battistella, S., Talarico, F.F., Brandmayr, T.Z. & Giulianini, P.G. (2008). Circulating haemocytes from larvae and adults of *Carabus* (*Chaetocarabus*) *lefebvrei* Dejean 1826 (Coleoptera, Carabidae): cell types and their role in phagocytosis after *in vivo* artificial non-self-challenge. *Micron,* 39, 552-558. https://doi. org/ 10. 1016/j.micron.2007.07.004

Gillespie, J.P., Kanost, M.R. & Trenczek, T. (1997). Biological mediators of insect immunity. *Annual Review of Entomology,* 42, 611-643. DOI: 10.1146/annurev.ento. 42.1.611

Giulianini, P.G., Bertolo, F., Battistella, S. & Amirante, G.A. (2003). Ultrastructure of the hemocytes of *Cetonischema aeruginosa* larvae (Coleoptera, Scarabaeidae): involvement of both granulocytes and oenocytoids in *in vivo* phagocytosis. *Tissue Cell*, 35, 243-251. https:// doi. org/10.1016/S0040-8166(03)00037-5

Glick D., Barth S. & Macleod KF (2010). Autophagy: cellular and molecular mechanisms. *Journal of Pathology*, 221, 3-12. https://doi.org/10.1002/path. 2697

Gorman, M.J., An, C. & Kanost, M.R. (2007). Characterization of tyrosine hydroxylase from *Manduca sexta*. *insect biochemistry and insect molecular biology*, 37, 1327-1337. DOI: 10.1016/j.ibmb.2007.08.006

Götz, P. & Boman, H.G. (1985). Insect immunity. In: "Comprehensive insect physiology, biochemistry and pharmacology"(Kerkut GA, Gilbert LI, Editors). pp. 453-487. Pergamon Press.

Gregoire, C. (1957). Studies by phase-contrast microscopy on distribution patterns of hemolymph coagulation in insects. *Smithsonian Institution Miscellaneous Collections*, 134, 1-35.

Gupta, A.P. (1979). Insect hemocytes: development, forms, functions, and techniques. Cambridge University Press., New York, 614 pp.

Gupta, A.P. (1985). Cellular Elements in the Hemolymph. In: "Comprehensive Insect Physiology Biochemistry Pharmacology" (Kerkut, G.A. & Gilbert, L.I., eds.). Pergamon Press, New York, pp: 400-451.

Gupta, A.P. & Sutherland, D.J. (1966). *In vitro* transformations of the insect plasmatocyte in some insects. *Journal of Insect Physiology,* 12, 1369-1375. https://doi.org/ 10. 1016/0022-1910(66)90151-X

Gupta, S.. Wang, Y. & Jiang, H. (2005). Purification and characterization of *Manduca sexta* prophenoloxidase-activating proteinase-1, an enzyme involved in insect immune responses. *Protein Expression and Purification,* 39, 261- 268. https:// doi. org/10. 1016/j.pep.2004.10.011

Halawa, S., Gaaboub, I., Gad, A.A. & El-Aswad, A.F. (2007). Effect of some insecticides on the haemolymph of desert locust *Schistocerca gregaria* Forskal. *Journal of Egyptian Society of Toxicology*, 36, 61-66.

Hamadah, Kh.Sh., Ghoneim, K.S., El-Hela, A.A., Amer, S.M. & Mohammad, A.A.1. (2013). Disturbed survival, growth and development of the desert locust *Schistocerca gregaria* by different extracts of *Azadirachta indica* (Meliaceae) and *Nigella sativa* (Ranunculaceae). *Egyptian Academic Journal of Biological Sciences, A.Entomology,* vol.6(2), 01 -21.

Hassan, H.A., Bakr, R.F.A., Abd El-Bar, M.M., Nawar, G.A. & Elbanna, H.M. (2013). Changes of cotton leaf worm haemocytes and esterases after exposure to compounds derived from urea and rice straw. Egypt.Acad.J. Biolog., 5(2), 35-48.

Haszcz, K. (2016). Impairing the insect immune system with plant-derived substances. M.Sc. Thesis, the Graduate College of Missouri State University, 43pp. http://bearworks. missouristate. edu/theses/ 2383

Hegedus, D., Erlandson, M., Gillott, C. & Toprak, U. (2009). New insights into peritrophic

matrix synthesis, architecture, and function. *Annual Review of Entomology,* 54, 285–302. DOI: 10.1146/annurev. ento. 54.110807. 090559

Hernandez, S., Lanz, H., Rodriguez, M.H., Torres, J.A., Martinez, P.A. & Tsutsumi, V. (1999). Morphological and cytochemical characterization of female *Anopheles albimanus* (Diptera: Culicidae) hemocytes. *Journal of Medical Entomology,* 36, 426-434. https://doi.org/10.1093/ jmedent/36.4.426

Hillyer, J.F. (2016). Insect immunology and hematopoiesis. *Developmental and Comparative Immunology,* 58,102-118. https://doi.org/10.1016/j.dci.2015.12.006

Hillyer, J.F. & Strand, M.R. (2014). Mosquito hemocyte-mediated immune responses. *Current Opinion in Insect Science,* 3:14-21. DOI: 10.1016/j.cois.2014.07.002

Hillyer, J.F., Schmidt, S.L. & Christensen, B.M. (2004). The antibacterial innate immune response by the mosquito *Aedes aegypti* is mediated by hemocytes and independent of Gram type and pathogenicity. *Microbes Infection,* 6, 448-459. https://doi. org/ 10.1016/j.micinf.2004.01.005

Hoffmann, J.A. (2003). The immune response of *Drosophila*. Nature, 426, 33–38.

Hoffmann, J.A., Reichhart, J.M. & Hetru, C. (1996). Innate immunity in higher insects. *Current Opinion in Immunology,* 8, 8-13.

Huang, J.F., Shui, K.J., Li, H.Y., Hu, M.Y. & Zhong, G.H. (2011). Antiproliferative effect of azadirachtin A on *Spodoptera litura* Sl-1 cell line through cell cycle arrest and apoptosis induced by up-regulation of p53. *Pesticide Biochemistry and Physiology,* 99, 16-24.

Huron, E.N., Abbas, A.A., Abd El-Hamid, N.A., Nada, M.S. & Amin, T.R. (2016). Toxicity and acute macromolecular abnormalities induced by some plant extracts against the Cowpea aphid, *Aphis carricivora* Koch. J. Plant Prot. and Path., Mansoura Univ. (Egypt), 7(7), 445-449.

Hussain, A. (2012). The effect of non-host plant volatiles on the reproductive behaviour of the Egyptian cotton leafworm, *Spodoptera littoralis*. M.Sc. Thesis, Swedish Univ. Agric.Sci., 40pp.

Hwang, S., Bang, K., Lee, J. & Cho, S. (2015). Circulating hemocytes from larvae of the Japanese rhinoceros beetle *Allomyrina dichotoma* (Linnaeus)(Coleoptera: Scarabaeidae) and the cellular immune response to microorganisms. *PLoS ONE,* 10(6), e0128519. doi:10.1371/ journal. pone.0128519

Imler, J.L. & Bulet, P. (2005). Antimicrobial peptides in *Drosophila*, structures, activities and gene regulation. *Chemical Immunology and Allergy,* 86, 1-21. DOI:10. 1159/ 000086648

Irving, P., Ubeda, J., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J., Hetru, C. & Meister, M. (2005). New insights into *Drosophila* larval haemocyte functions through genome wide analysis. *Cellular Microbiology,* 7(3), 335-350. https://doi.org/10.1111/ j.1462-5822.2004. 00462.x

Islam, A. & Roy, S. (1982), Diurnal rhythm of hemocyte population in an insect *Schizodactylus monstresus* Drury (Orthoptera). *Experientia,* 38, 567-569. DOI: 10. 1007/BF02327052

İzzetoğlu, S. & Karaçali, S. (2010). A novel site for hematopoietic organ in *Bombyx mori* L. (Lepidoptera: Bombycidae). The Journal of Faculty of Veterinary Medicine, Kafkas University, Turkey, 16 (Suppl-B), 243-247.

Jalali, J. & Salehi, R. (2008), The hemocyte types, differential and total count in *Papilio demoleus* L. (Lepidoptera: Papilionidae) during post-embryonic development. *Munis Entomology and Zoology,* 1, 199-216.

James, R.R. & Xu, J. (2012). Mechanisms by which pesticides affect insect immunity. *Journal of Invertebrate Pathology*, 109(2), 175-182. https://doi.org/10.1016/j.

jip.2011.12.005

Janeway, C.A. Jr. & Medzhitov, R. (2002). Innate immune recognition. Annual Review of Immunology, 20, 197-216. https://doi.org/10.1146/annurev. immunol.20. 083001. 084359.

Jeyasankar, A. & Jesudasan, R.W.A. (2005). Insecticidal properties of novel botanicals against a few lepidopteran pests. *Pestology*, 29, 42-44.

Jiang, H., Vilcinskas, A. & Kanost, M.R. (2010). Immunity in lepidopteran insects. *Advanced Expermintal Medicine and Biology*, 708, 181-204. https://doi.org/10. 1007/978-1-4419-8059-5_10

John, P.A. & Ananthakrishnan, T.N. (1995). Impact of azadirachtin on the haemodynamics of *Cyrtacanthacris tatarica* L. (Acrididae: Orthoptera). *Journal of Entomological Research*, 19(4), 285-290. http://oar.icrisat.org/id/eprint/6879

Jones, J.C. (1959). A phase contrast study of the blood cells in *Prodenia* larvae (Order Lepidoptera). *Quarterly Journal of Microscopical Science,* 100, 17-23.

Jones, J.C. (1962). Current concepts concerning insect haemocytes. *Integrative and Comparative Biology*, 2(2), 209-246. https://doi. org/ 10. 1093/icb/2.2.209

Jones, J.C. (1967a). Changes in the haemocyte picture of *Galleria mellonella* (L.). *Biol.ogy Bulletin*, 132, 211-221. DOI: 10.2307/1539889

Jones, J.C. (1967b). Normal differential count of haemocytes in relation to ecdysis and feeding in *Rhodnius prolixus*. *Journal of Insect Physiology,* 13, 1133-1143. https://doi. org/10.1016/0022-1910(67)90087-X

Jones, J.C. & Tauber, O.E. (1954). Abnormal haemocytes in mealworm (*Tenebrio molitor* L.). *Annals of Entomological Society of America*, 47(3), 428-444. https://doi.org/10. 1093/aesa/47.3.428

Jones, J.C. & Liu, D.P. (1961). Total and differential haemocyte counts of *Rhodnius prolixus* Stal. *Bulletin of Society for Science and Technology*, *India*, 7, 166.

Kaidi, N., Amroun, C., Hocine, D., Doumandji, S. & Ghezali, D. (2017). Biological activity of *Calotropis procera* Ait on mortality and haemogram of *Schistocerca gregaria* (Forskal, 1775) and *Locusta migratoria* (Linné, 1758). *Advances in Environmental Biology,* 11(4), 37-45.

Kalyani, S.S. & Holihosur, R.S.N. (2015). Toxic effect of crude aqueous leaf extracts of *Clerodendron inerme*, on the total haemocyte count of sixth instar larva of *Helicoverpa armigera* (H). *International Journal for Innovative Research in Science & Technology*, 1(12), 221-224.

Kanost, M.R. & Gorman, M.J. (2008). Phenoloxidases in insect immunity. In: "Insect immunology". Pp: 69-96. Academic Press/Elsevier, San Diego.

Kanost, M.R., Jiang, H. & Yu, X.Q. (2004). Innate immune responses of a lepidopteran insect, *Manduca sexta*. *Immunological Reviews,* 198, 97-105. https://doi. org/ 10. 1111/j.0105-2896.2004. 0121.x

Khafagi, W.E. & Hegazi, E.M. (2001). Effect of juvenile hor-mones and precocenes on the immune response of *Spodoptera littoralis* larvae to supernumerary larvae of the solitary parasitoid *Microplitis rufiventris* Kok. *Journal of Insect Physiology,* 47, 1249-1259. https://doi.org/ 10. 1016/S0022-1910(01)00110-X

Khanikor, B. & Bora, D. (2012). Effect of plant-based essential oil on immune response of silkworm, *Antheraea assama* Westwood (Lepidoptera: Saturniidae). International *Journal of Industrial Entomology*, 25(2), 139-146. DOI:10.7852/ ijie. 2012.25.2.139

Khosravi, R., Sendi, J.J., Brayner, F.A., Alves, L.C. & Feitosa, A.P.S. (2016). Hemocytes of the rose sawfly *Arge ochropus* (Gmelin) (Hymenoptera: Argidae). *Neotropical Entomology*, 45(1), 11pp. DOI: 10.1007/s13744-015-0339-9

Kim, E.J., Rhee, W.J. & Park, T.H. (2001). Isolation and characterization of an apoptosis-inhibiting component from the hemolymph of *Bombyx mori*. Biochemical and Biophysical *Research Communications,* 285(2), 224-228. https://doi.org/ 10. 1006/bbrc.2001.5148

Kohan, R. & Sendi, J.J. (2013). Immune responses of elm leaf beetle *Xanthogaleruca luteola* Mull. (Col: Chrysomellidae) to *Beaveria bassiana* and *Artemisia annua* essential oil. *SOAJ Entomological Studies,* 2, 16-25.

Kohlmaier, A. & Edgar, B.A. (2008). Proliferative control in *Drosophila* stem cells. *Current Opinion in Cell Biology,* 20, 699-706. https://doi.org/10. 1016/j.ceb. 2008. 10.002

Koodalingam, A., Mullainadhan, P. & Arumugam, M. (2009). Antimosquito activity of aqueous kernel extract of soapnut *Sapindus emarginatus*: impact on various developmental stages of three vector mosquito species and non-target aquatic insects. *Parasitology Research,* 105, 1425-1434. https://doi.org/10. 1007/s00436-009-1574-y

Koodalingam, A., Mullainadhan, P. & Arumugam, M. (2011). Effects of extract of soapnut *Sapindus emarginatus* on esterases and phosphatases of the vector mosquito, *Aedes aegypti* (Diptera: Culicidae). *Acta Tropica,* 118, 27-36. https://doi.org/ 10.1016/ j.actatropica. 2011.01.003

Koodalingam, A., Mullainadhan, P. & Arumugam, M. (2013). Immuno-suppressive effects of aqueous extract of soapnut *Sapindus emarginatus* on the larvae and pupae of vector mosquito, *Aedes aegypti*. *Acta Tropica,* 126, 249-255. https://doi.org/10. 1016/j.actatropica. 2013.02.021

Koodalingam, A., Deepalakshmi, R., Ammu, M. & Rajalakshmi, A. (2014). Effects of NeemAzal on marker enzymes and hemocyte phagocytic activity of larvae and pupae of the vector mosquito *Aedes aegypti*. *Journal of Asia-Pacific Entomology,* 17(2), 175-181. https://doi.org/10.1016/j.aspen.2013.12.007

Koul, O. (2004). Biological activity of volatile D-n-Propyl Difulside from seeds of neem, *Azadirachta indica* (Meliaceae), to two species of stored grain pests, *Sitophilus oryzae* (L.) and *Tribolium oryzae* (Herbst). *Journal of Economic Entomology,* 97, 1142-1147. https://doi. org/10.1093/jee/97.3.1142

Krams, I., Kecko, S., Kangassalo, K., Moore, F.R., Jankevics, E., Inashkina, I., Krama, T., Lietuvietis, V., Meija, L. & Rantala, M.J. (2014). Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth *Galleria mellonella*. *Insect Science,* 22, 431–439. https://doi.org/10. 1111/1744-7917.12132

Kuballa, P., Nolte, W.M., Caastoreno, A.B. & Xavier, R.J. (2012). Autophagy and the immune system. *Annual Review of Immunology,* 30, 611-646. https://doi.org/10. 1146/ annurev-immunol-020711-074948

Kurihara, Y., Shimazu, T. & Wago, H. (1992). Classification of hemocytes in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae): I. Phase microscopic study. *Applied Entomology and Zoology,* 27(2), 225-235. DOI: 10.1303/aez.27.225

Kurt, D. & Kayis, T. (2015). Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*. *Turkish Journal of Zoology,* 39, 452-457.

Kurtz J. (2002). Phagocitosis by invertebrate hemocytes: causes of individual variation in *Panorpia vulgaris* scorpion flies. *Microscopy Research and Technique,* 57(6), 456-68. doi: http://doi.org/dcws99.

Kwon, H., Bang, K. & Cho, S. (2014). Characterization of the hemocytes in larvae of *Protaetia brevitarsis seulensis*: involvement of granulocyte-mediated phagocytosis. *PLoS ONE,* 9(8), e103620. doi: 10.1371/journal.pone.0103620

Lackie, A.M. (1988). Haemocyte behaviour. Adv. Insect Physiol., 21, 85-178.

https://doi.org/10.1016/S0065-2806(08)60123-X

Lampert, E. (2012): Influences of plant traits on immune responses of specialist and generalist herbivores. *Insects,* 3: 573-592. Doi: 10.3390/insects3020573

Lamprou, I., Mamali, I., Dallas, K., Fertakis, V., Lampropoulou, M. & Marmaras, V.J. (2007). Distinct signalling pathways promote phagocytosis of bacteria, latex beads and lipopolysaccharide in medfly haemocytes. *Immunology,* 121, 314-327. DOI: 10.1111/j.1365-2567.2007. 02576.x

Lavine, M.D. & Strand, M.R. (2002). Insect haemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology, 32*2, 1295-1309. https://doi.org/10.1016/ S0965-1748(02)00092-9

Levin, D.M., Breuer, L.N., Zhuang, S., Anderson, S.A., Nardi, J.B. & Kanost, M.R. (2005). A Hemocyte-specific integrin required for hemocytic encapsulation in the tobacco hornworm, *Manduca sexta*. *Insect Biochemistry and Molecular Biology,* 35 (5), 369-380. https://doi.org/10.1016/j.ibmb.2005.01.003

Ling, E. & Yu, X.Q. (2006). Hemocytes from the tobacco hornworm *Manduca sexta* have distinct functions in phagocytosis of foreign particles and self-dead cells. *Developopmental Compatative Immunology*, 30, 301- 309.

Ling, E., Shirai, K., Kanekatsu, R. & Kiguchi, K. (2005). Hemocyte differentiation in the hematopoietic organs of the silkworm, *Bombyx mori*: prohemocytes have the function of phagocytosis. *Cell Tissue Research,* 320, 535-543. https://doi.org/10. 1007/s00441-004-1038-8

Liu, F., Huang, W., Wu, K., Qiu, Z., Huang, Y. & Ling, E. (2017). Chapter Seven - Exploiting Innate Immunity for Biological Pest Control. *Advances in Insect Physiology,* 52, 199-230. https://doi.org/10.1016/bs.aiip.2017.02.001

Lokesh, K.V., Kanmani, S., Adline, J.D., Raveen, R., Samuel, T., Arivoli, S. & Jayakumar, M. (2017). Adulticidal activity of *Nicotiana tabacum* Linnaeus (Solanaceae) leaf extracts against the sweet potato weevil *Cylas formicarius* Fabricius 1798 (Coleoptera: Brentidae). *Journal of Entomology and Zoology Studies,* 5(5), 518-524.

Lowenberger, C. (2001). Innate immune response of *Aedes aegypti*. *insect biochemistry and insect molecular biology*, 31, 219-229. https://doi.org/10.1016/ s0965-1748(00)00141-7

Lynn, D.C. & Vinson, S.B. (1977), Effects of temperature, host age and hormones upon the encapsulation of *Cardiochilis nigriceps* eggs by *Heliothis* sp. *Journal of Invertebrate Pathology,* 29, 50-55. https://doi.org/10.1016/ 0022-2011(77)90171-9

Manachini, B., Arizza, V., Parrinello, D. & Parrinello, N. (2011). Hemocytes of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) and their response to *Saccharomyces cerevisiae* and *Bacillus thuringiensis*. *Journal of Invertebrate Pathology,* 106(3), 360-365. DOI: 10.1016/j.jip.2010.12.006

Manogem, E.M., Arathi, S. & Shony, U. (2015). A study on the haemocytes profile of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *International Journal of Pure aand Applied Bioscience,* 3(5), 113-120. DOI: http:// dx. doi. org/10. 18782/2320-7051.2109

Manogem, E.M., Cheruparambath, P., Shibi, P., Arathi, S. & Banu, A. (2016). Effect of chitin synthesis inhibitor, Flufenoxuron, on haemocytes of *Spodoptera mauritia* (Boisd.)(Lepidoptera: Noctuidae). International Journal of Plant, *Animal and Environmental Sciences,* 6(1), 68-75.

Marciniak, P., Adamski, Z., Bednarz, P., Slocinska, M., Ziemnicki, K., Lelario, F., Scrano, L. & Bufo, S.A. (2010). Cardioinhibitory properties of potato glycoalkaloids in beetles. *Bulletin of Environmental Contamination and Toxicology,* 84(2), 153-156.

https://doi.org/10.1007/s00128-009-9921-3

Marmaras, V.J. & Lampropoulou, M. (2009), Regulators and signalling in insect haemocyte immunity. *Cell Signal*, 21, 186-195. https://doi.org/10.1016/j. cellsig. 2008.08.014

Meister, M. (2004), Blood cells of *Drosophila*: cell lineages and role in host defence. *Current Opinion Immunology*, 16, 10-15. https://doi.org/10.1016/j.coi.2003.11.002

Meister, M. & Lagueux, M. (2003). *Drosophila* blood cells. *Cell Microbiology*, 5, 573-580. DOI: 10.1046/j.1462-5822.2003. 00302.x

Meister, M., Hetru, C. & Hoffmann, J.A. (2000). The antimicrobial host defense of *Drosophila*. In: "Current Topics in Microbiology and Immunology-Origin and Evolution of the Vertebrate Immune System"(Du Pasquier, L. and Litman, G.W., eds.). vol. 248, 17-36. Springer-Verlag, Berlin.

Merchant, D., Ertl, R.L., Rennard, S.I., Stanley, D.W. & Miller, J.S. (2008). Eicosanoids mediate insect hemocyte migration. *Journal of Insect Physiology*, 54, 215-221. https://doi.org/10.1016/j.jinsphys.2007.09.004

Miles, M. & Lysandrou, M. (2002). Evidence for negative cross resistance to insecticides in field collected *Spodoptera littoralis* (Boisd.) from Lebanon inlaboratory bioassays. *Mededelingen (Rijksuniversiteitte Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen)*, 67, 665.

Millar, D.A. & Ratcliffe, N.A. (1989). The evolution of blood cells: facts and enigmas. *Endeavour, 13*, 72-77. https://doi.org/10.1016/0160-9327(89)90005-7

Morgenthaler, P.W. (1953). Blutuntersuchungen bei Bienen. Mitteil. Schweiz. *Entomology Gesell*, 26, 245-257.

Morya, K., Pillai, S. & Patel, P. (2010). Effect of powdered leaves of *Lantana camara, Clerodendrum inerme* and *Citrus limon* on the rice moth, *Corcyra cephalonica*. *Bulletin of Insectology*, 63(2), 183-189.

Mosallanejad, H. & Smagghe, G. (2009). Biochemical mechanisms of methoxyfenozide resistance in the cotton leafworm *Spodoptera littoralis*. *Pest Management Science*, 65, 732-736. DOI: 10.1002/ps.1753

Mullen, L. & Goldsworthy, G. (2003). Changes in lipophorins are related to the activation of phenoloxidase in the haemolymph of *Locusta migratoria* in response to injection of immunogens. *insect biochemistry and insect molecular biology*, 33, 661-670. https://doi.org/10.1016/S0965-1748(03)00045-6

Muta, T. & Iwanaga, S. (1996). The role of hemolympyh coagulation in innate immunity. *Current Opinion Immunology*, 8, 41-47. https://doi.org/10.1016/S0952-7915 (96) 80103-8

Nappi, A.J. (1970). Haemocytes of larvae of *Drosophila euronotus* (Diptera: Drosophilidae). *Annals of Entomological Society of America*, 63, 1217–1224. https://doi.org/10.1093/aesa/63.5.1217

Nappi, J.A. (1974). Insect haemocytes and the problem of host recognition of foreigners. In: "Contemporary Topics in Immuonology-Invertebrate immunity"(Cooper, E.L., ed.), IV, 207-224. Plenum Press, New York and London.

Nardi, J.B. (2004). Embryonic origins of the two main classes of hemocytes granular cells and plasmatocytes in *Manduca sexta*. *Development, Genes and Evolution*, 214(1), 19-28. https://doi.org/10. 1007/s00427-003-0371-3

Nardi, J.B., Gao, C. & Kanost, M.R. (2001). The extracellular matrix protein lacunin is expressed by a subset of hemocytes involved in basal lamina morphogenesis. *Journal of Insect Physiology*, 47, 997-1006. https://doi.org/10.1016/S0022-1910 (01) 00074-9

Nardi, J.B., Pilas, B., Ujhelyi, E., Garsha, K. & Kanost, M.R. (2003). Hematopoietic organs

of *Manduca sexta* and hemocyte lineages. *Development Genes and Evolution,* 213(10), 477-491. https://doi.org/ 10.1007/s00427-003-0352-6

Niroula, S.P. & Vaidya, K. (2004). Efficacy of some botanicals against Potato tuber moth, *Phthorimaea operculella* (Zeller, 1873). *Our Nature,* 2, 21-25. doi:10.3126/on. v2i1.320

Nishi, S.P. (1982). Observation on the haemocytes of different stages of *Spodoptera litura* (Noctuidae: Lepidoptera) in relation to application of Betaecdysone. M.Sc. Thesis, Aligarh Muslim University, Aligarh, India, 172pp.

Ntalli, N., Kopiczko, A., Radtke, K., Marciniak, P., Rosinski, G. & Adamski, Z. (2014). Biological activity of *Melia azedarach* extracts against *Spodoptera exigua*. *Biologia,* 69(11), 1606-1614. https://doi.org/10.2478/s11756-014-0454-9

Oczypok, E.A., Oury, T.D. & Chu, C.T. (2013). It's a cell-eat-cell world, Autophagy and phagocytosis. *American Journal of Pathology*, 182, 612-622. https://doi.org/10. 1016/j. ajpath.2012.12.017

Okazaki, T., Okudaira, N., Iwabuchi, K., Fugo, H. & Nagai, T. (2006). Apoptosis and adhesion of hemocytes during molting stage of silkworm, *Bombyx mori*. *Zoology Science,* 23, 299-304. https://doi.org/10. 2108/zsj.23.299

Ordas, M.C., Ordas, A., Belosa, C. & Figueras, A. (2000). Immune parameters in carpet shell clams naturally infected with *Perkinsus atlanticus*. *Fish Shellfish Immunology,* 10(7), 597-609. https://doi. org/10.1006/fsim.2000.0274

Orser, W.B. & Brown, A.W.A. (1951). The effect of insecticides on the heartbeat of *Periplaneta*. *Canadian Journal of Zoology,* 29(1), 54-64. DOI: 10.1139/z51-005

Padmaja, P.G. & Rao, P.J. (2000). Effect of plant oils on the total haemocyte count (THC) of final instar larvae of *Helicoverpa armigera* Hübner. *Pesticide Research Journal,* 12(1), 112-116.

Pandey, J.P. (2004). Studies on stress induced haematological changes in *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae) and *Danais chryssipus* (Lepidoptera: Nymphalidae). Ph.D.Thesis, V.B.S. Purvanchal University, Jaunpur, India.

Pandey, J.P. & Tiwari, R.K. (2011). Neem based insecticides interaction with development and fecundity of red cotton bug, *Dysdercus cingulatus* Fab. *International Journal of Agricultural Research,* 6(4), 335-346. DOI:10.3923/ ijar.2011.335.346

Pandey, J.P., Upadhyay, A.K. & Tiwari, R.K. (2007). Effect of some plant extracts on haemocyte count and moulting of *Danais chrysippus* larvae. *Journal of Advanced Zoology*, 28, 14-20. DOI: 10.3923/je.2012.23.31

Pandey, J.P., Tiwari, R.K. & Kumar, D. (2008). Reduction in hemocyte mediated immune response in *Danais chrysippus* following treatment with neem-based insecticides. *Journal of Entomology,* 5, 200-206. DOI: 10.3923/ je.2008.200.206

Pandey, J.P., Mishra, P.K., Kumar, D., Singh, B.M.K. & Prasad, B.C. (2010). Effect of temperature on hemocytic immune responses of tropical tasar silkworm, *Antheraea mylitta* D. *Research of Journal Immunology,* 3, 169-177. DOI:10.3923/rji. 2010. 169. 177

Pandey, S., Pandey, J.P. & Tiwari, R.K. (2012). Effect of botanicals on hemocytes and molting of *Papilio demoleus* larvae. *Journal of Entomology,* 9(1), 23-31. DOI:10. 3923/ je.2012.23.31

Pathak, S.C. & Kulshreshtha, V. (1993). Variation in haemocyte types with reference to reproduction activity in *Blatella germanica* L. (Dictyoptera: Blattidae) and the occurrence of undescribed haemocyte types in some adult stages. *Entomon,* 18, 119-125.

Patton, R.L. & D.S. Sarkaria, (1958). The gross pathology of the American cockroach following injection with organic solvents. *Journal of Economic Entomology,* 51,

663-665. https:// doi. org/10.1093/jee/51.5.663

Pendland, J.C. & Boucias, D.G. (1996). Phagocytosis of lectin-opsonized fungal cells and endocytosis of the ligand by insect *Spodoptera exigua* granular hemocytes: an ultrastructural and immunocytochemical study. *Cell and Tissue Research,* 285, 57-67. https:// doi. org/10.1007/s004410050620

Piazza, N. & Wessells, R.J. (2011). *Drosophila* models of cardiac disease. *Progress in Molecular Biology and Translational Science,* 100,155–210.

Qadri, S.S.H. & Narsaiah, J. (1978). Effect of azadirachtin on the moulting processes of last instar nymphs of *Periplaneta americana* (L.). *Indian Journal of Expermintal Biology,* 16, 1141-1143.

Qamar, A. & Jamal, K. (2009). Differential haemocyte counts of 5th instar nymphs and adults of *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide. *Biology and Medicine,* 1(2),116-121.

Raina, A.K. (1976). Ultrastructure of the larval hemocytes of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *International Journal of Insect Morphology and Embryology,* 5(3), 187-195. https://doi.org/10.1016/0020-7322(76)90003-9

Rantala, M.J., Vainikka, A. & Kortet, R. (2003). The role of juvenile hormone in immune function and pheromone production trade-offs: a test of the immunocompetence handicap principle. *Proceedings of the Royal Society B: Biological Sciences*, 270, 2257-2261. DOI: 10.1098/rspb.2003.2472

Rao, P.C.G., Ray, A. & Ramamurty, P.S. (1984). Effect of ligation and ecdysone on total haemocyte count in the tobacco caterpillar, *Spodoptera litura* (Noctuidae: Lepidoptera). *Canadian Journal of Zoology,* 62, 1461-1463. https://doi.org/10.1139/z84-211

Ratcliffe, N.A. (1985). Invertebrate immunity- a primer for the non-specialist. *Immunology Letters,* 10, 253-270. https://doi.org/10.1016/0165-2478(85)90100-2

Ratcliffe, N.A. & George, S.J. (1976). Cellular defense reactions of insect haemocytes *in vivo*: nodule formation and development in *Galleria mellonella* and *Pieris brassicae* larvae. *Journal of Invertebrate Pathology,* 28, 373- 382. https://doi.org/10.1016/0022-2011(76)90013-6

Ratcliffe N.A. & Gagen S.J. (1977). Studies on the *in vivo* cellular reactions of insects: an ultrastructural analysis of nodule formation in *Galleria mellonella*. *Tissue Cell,* 9, 73-85. DOI:10.1016/0040-8166 (77) 90050-7

Ratcliffe, N.A. & Rowley, A.F. (1987). Insect response to parasites and other pathogens. In: "Immunology, Immunoprophylaxis and Immunotherapy of Parasitic Infections"(Soulsby, E.J.L., ed.). pp: 271–332, Boca Raton, FL, USA, CRC Press.

Redfern, R.E., Kelly, T.J. & Hayes, D.K. (1982). Ecdysteroid titers and moulting aberrations in last stage of O*ncopeltus* nymphs treated with insect growth regulators. *Pesticide Biochemistry and Physiology,* 18, 351-356. https://doi.org/10.1016/0048-3575(82)90076-1

Reed, E. & Majumdar, S.K. (1998). Differential cytotoxic effects of azadirachtin on *Spodoptera frugiperda* and mouse cultured cells. *Entomologia Experimentalis et Applicata,* 89(3), 215-221. https:// doi. org/10.1046/j.1570-7458.1998. 00402.x

Reeson, A.F., Wilson, K., Gunn, A., Hails, R.S. & Goulson, D. (1998). Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proceedings of the Royal Society of London B: Biological Sciences,* 265, 1787-1791. https://www.jstor.org/stable/51260

Relyea, R.A. (2009). A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia,* 159, 363-376. DOI:

10.1007/s00442-008-1213-9

Ribeiro, C. & Brehelin, M. (2006). Insect haemocytes: what type of cell is that?. *Journal of Insect Physiology*, 52, 417- 429. https://doi.org/10.1016/j. jinsphys.2006.01.005

Ribeiro, S., Guilhermino, L., Sousa, J.P. & Soares, A.M.V.M. (1999). Novel bioassay based on acetylcholinesterase and lactate dehydrogenase activities to evaluate the toxicity of chemicals to soil isopods. *Ecotoxicology and Environmental Safety*, 44, 287-293. https://doi.org/ 10. 1006/eesa.1999.1837

Rizk, S.A. (1991). Effect of gamma radiation and some insecticides on the cotton leaf worm *Spodoptera littoralis* (Boisd.). M.Sc. Thesis, Faculty of Science, Cairo University, Egypt.

Rizk, S.A., El–Halfawy, N.A. & Salem, H.M. (2001). Toxicity and effect of Margosan–O and azadirachtin on haemocytes of *Spodoptera littoralis* (Boisd.) larvae. *Bulletin of the Entomological Society of Egypt / Economic series*,.28, 39-48.

Romao, S. & Munz, C. (2011). Autophagy of pathogens alarms the immune system and participates in its effector functions. *Swiss Medical Weekly*, 141, W13198. DOI:10.4414/smw.2011.13198

Sabri, M.A. & Tariq, B. (2004). Toxicity of some insecticides on the haemocytes of red pumpkin beetle, *Aulacophora foveicollis* Lucas. *Jornal of Pakistan Entomology*, 26, 109-114.

Sabry, K.H. & Abdel-Aziz, N.F. (2013). Resistance and enzyme assessment of the pink bollworm, *Pectinophora gossypiella* (Saunders) to Spinosad. *Journal of Animal & Plant Sciences*, 23(1), 136-142.

Sadeghi, R., Raeisi, N.H. & Jamshidnia, A. (2017). Immunological responses of *Sesamia cretica* to *Ferula ovina* essential oil. *Journal of Insect Science*, 17(1), 1–5. DOI | 10.1093/jisesa/iew124

Sadek, M.M. (2003). Antifeedant and toxic activity of Adhatoda vesica leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). *Journal of Applied Entomology*, 127, 396-404. https://doi.org/10.1046/j.1439-0418.2003.00775.x

Sahayaraj, K. & Kombiah, P. (2010). Insecticidal activities of neem gold on banana rhizome weevil (BRW), *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Journal of Biopesticides*, 3(1 Special Issue), 304 -308.

Saito, T. & Iwabuchi, K. (2003)0 Effect of bombyxin-II, an insulin-related peptide of insects, on *Bombyx mori* hemocyte division in single-cell culture. *Applied Entomology and Zoology*, 39, 583-588. https:// doi.org/ 10.1303/aez.2003.583

Salama, M.A., Abd El-Baki, M.A., El-Naggar, J.B.A. & El-Naggar, E.Y. (2013). Efficiency of some insecticides sequence on cotton bollworms and histopathological effects of some biocides on pink bollworm larvae. *Egyptian Journal of Agricultural Research*, 91(2), 429-448.

Salehzadeh, A., Akhkha, A., Cushley. W., Adams, R.L., Kusel, J.R. & Strang, R.H. (2003). The antimitotic effect of the neem terpenoid azadirachtin on cultured insect cells. *Insect Biochemstry and Molecular*, 33, 681-689. https://doi.org/10.1016/S0965-1748(03)00057-2

Saxena, B.P. & Tikku, K. (1990). Effect of plumbagin on hemocytes of *Dysdercus koenigii* F. Proc. *Indian Academy Sciences (Animal Science)*, 99(2), 119-124.

Schmid-Hempel, P. (2005). Evolutionary ecology of insect immune defenses. *Annual Review of Entomology*, 50, 529-551. https://doi.org/10.1146/annurev.ento.50. 071803.130420

Schmidt, O., Theopold, U. & Strand, M.R. (2001). Innate immunity and evasion by hymenopteran endoparasitoids. *BioEssays*, 23, 344-351. DOI: 10.1002/bies.1049

Schmit, A.R. & Ratcliffe, N.A. (1977). The encapsulation of foreign tissue implants in

Galleria mellonella larvae. *Journal of Insect Physiology,* 23, 175-184. https://doi.org/10. 1016/0022-1910(77)90027-0

Schmutterer, H. (1990a). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology,* 35, 271- 297. https://doi.org/ 10.1146/annurev.en.35.010190.001415

Schmutterer, H. (1990b). Insektizide aus dem Niembaum *Azadirachta indica*. Sanfte Chemie Fur den integrierten Pflanzenschutz in Entwicklungs- and industrielandern. *Plits,* 8(2), 57-71.

Sendi, J.J. & Salehi, R. (2010). The effect of methoprene on total hemocyte counts and histopathology of hemocytes in *Papilio demoleus* L. (Lepidoptera). *Munis Entomology and Zoology,* 5(1), 240-246.

Shaalan, E., Canyon, D., Younes, M., Abdel-Wahab, H. & Mansour, A. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment International,* 31, 1149-1166. https://doi.org/10.1016/j.envint.2005.03.003

Shapiro, M., Stock, R.D. & Ignoffo, C.M. (1969). Hemocyte changes in larvae of the bollworm, *Heliothes zea*, infected with a nucleopolyhedrosis virus. *Journal of Invertebrate Pathology,* 14, 28-30. https://doi.org/10.1016/0022-2011(69)90006-8

Sharma, P.R., Sharma, O.P. & Saxena, B.P. (2001). Ultrastructureof the haemocytes of the tobacco armyworm, *Spodoptera litura* Fab. (Lerpidoptera: Noctuidae). *Biologia Bratislava*, 56(3), 277–285.

Sharma, P.R., Sharma, O.P. & Saxena, B.P. (2003). Effect of neem glod on hemocytes of the tobacco armyworm, *Spodoptera littura* (Fabricius) (Lepidoptera: Noctuidae). *Current Science,* 84(5), 690-695. https://www.jstor. org/stable/24108506

Sharma, P.R., Sharma, O.P. & Saxena, B.P. (2008). Effect of sweet flag rhizome oil (*Acorus calamus*) on hemogram and ultrastructure of hemocytes of the tobacco armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Micron,* 39, 544-551. https://doi.org/ 10.1016/ j.micron. 2007. 07.005

Sharma, N.K., Ahirwar, D., Jhade, D. & Gupta, S. (2009). Medicinal and Phamacological Potential of *Nigella sativa*: *A Review. Ethnobotanical Review*, 13, 946-955.

Shaurub, E.H. (2012). Immunonodulation in insects post-treatment with abiotic agents: a review. *European Journal of Entomology*, 109, 303-316. DOI: 10.14411/ eje. 2012. 040

Shaurub, E.H. & Sabbour, M.M. (2017). Impacts of pyriproxyfen, flufenoxuron and acetone extract of *Melia azedarach* fruits on the hemolymph picture of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Advances in Agricultural Science,* 5(2), 1-9. https://aaasjournal.org/submission/index. php/ aaas/article/view/11

Shaurub, E.H., Abd El-Meguid, A. & Abd El-Aziz, N.M. (2014). Quantitative and ultrastructural changes in the haemocytes of *Spodoptera littoralis* (Boisd.) treated individually or in combination with *Spodoptera littoralis* multicapsid nucleopolyhedrovirus (SpliMNPV) and azadirachtin. *Micron,* 65, 62–68. DOI: 10. 1016/j. micron.2014.04.010

Shu, B., Wang, W., Hu, Q., Huang, J., Hu, M. & Zhong, G. (2015). A comprehensive study on apoptosis induction by azadirachtin in *Spodoptera frugiperda* cultured cell line Sf9. *Archives of Insect Biochemistry*, 89, 153-168. DOI: 10.1002/arch.21233

Shull, W.E., Riley, M.K. & Richardson, C.H. (1932). Some effects of certain toxic gases on the blood of the cockroach, *Periplaneta orientalis* (L.). *Journal of Economic Entomology*, 25, 1070-1072. https://doi.org/10.1093/jee/25.5.1070

Siddiqui, M.I. & Al-Khalifa, M.S. (2014). Review of haemocyte count, response to chemicals, phagocytosis, encapsulation and metamorphosis in insects. *Italian*

Journal of Zoology, 81(1), 2-15. doi: 10.1080/11250003.2013.858780

Silva, J.E.B., Boleli, I.C. & Simoes, Z.L.P. (2002). Hemocyte types and total and differential counts in unparasitized and parasitized *Anastrepha obliqua* (Diptera, Tephritidae) larvae. *Brazilian Journal of Biology*, 62(4a), 689-699. http://dx.doi.org/ 10.1590/S1519-69842002000400017

Sinclair, B.J., Ferguson, L.V., Salehipour-Shirazi, G. & Macmillan, H.A. (2013). Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integrative and Comparative Biology*, 53, 545–556. DOI: 10.1093/icb/ict004

Singh, G.P., Zeya, S.B., Srivastava, A.K., Prakash, B., Ojha, N.G. & Suryanarayana, N. (2008). Susceptibility of three Eco-races of tropical tasar silkworm to *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV). *Caspian Journal of Environmental Science*, 6, 161-165.

Soares, T., Cavalcanti, M.G.S., Ferreira, F.R.B., Cavalcanti M.S., Alves L.C., Brayner, F.A. & Paiva, P.M.G. (2013). Ultrastructural characterization of the hemocytes of *Lasiodora* sp. (Koch, 1850) (Araneae: Theraphosidae). *Micron*, 48, 11-16. doi:10.1016/j.micron. 2013.02.002

Sorrentino, R.P., Carton, Y. & Govind, S. (2002). Cellular immune responses to parasite infection in the *Drosophila* lymph gland is developmentally regulated. *Developmental Biology*, 243, 65-80. https://doi.org/10. 1006/dbio.2001.0542

Strand, M.R. (2008). The insect cellular immune response. *Insect Sciences*, 15, 1-14. https://doi.org/ 10.1111/j.1744-7917.2008. 00183.x

Strand, M.R. & Pech, L.L. (1995). Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology*, 40, 31-56. https://doi.org/10.1146/ annurev.en.40.010195.000335

Suhail, A., Gogi, M.D., Arif, M.J., Rana, M.A. & Sarfraz, M. (2007). Effect of various treatment of azadirachtin, spinosad and abamectin on the haemogram of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Pakistan Entomologist*, 29(2), 151-164.

Tavares, W.S., Cruz, I., Petacci, F., Assis Junior, S.L., Freitas, S., Zanuncio, J.C. & Serrao, J.E. (2009). Potential use of Asteraceae extracts to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and selectivity to their parasitoids *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and *Telenomus remus* (Hymenoptera: Scelionidae). *Industrial Crops and Products*, 30: 384-388. https://doi. org/10.1016/j.indcrop.2009.07.007

Tettamanti, G., Cao, Y., Feng, Q., Grimaldi, A. & Eguileor, M (2011). Autophagy in Lepidoptera: more than old wine in new bottle. *Invertebrate Survival Journal*, 8(1), 5-14.

Theopold, U., Schmidt, O., Soderhall, K. & Dushay, M.S. (2004). Coagulation in arthropods: defense, wound closure and healing. *Trends Immunology*, 25, 289-294. https://doi.org/10.1016/j.it.2004.03.004

Tikku, K., Saxena, B.P., Satti, N.K. & Suri, K.A. (1992). Plumbagin-induced ultrastructural haemocytic response of *Dysdercus koenigii* (F.). *Insect Science and its Application*, 13(6), 787-791. DOI: 10.1017/S1742758400008109

Tiwari, R.K., Pandey, J.P. & Salehi, R. (2002). Haemopoietic organs and effect of their ablation on total haemocyte count in lemon butterfly, *Papilio demoleus* L. *Indian Expermental Biology*, 40(10), 1202-1205.

Tiwari, R.K., Pandey, J.P. & Kumar, D. (2006). Effects of neem-based insecticides on metamorphosis, haemocytes count and reproductive behavior in red cotton bug, *Dysdercus koenigii* fabr (Heteroptera: Pyrrhocoridae). *Journal of Entomology and*

Zoology Studies, 31, 267-275.

Tojo, S., Naganuma, F., Arakawa, K. & Yokoo, S. (2000). Involvement of both granular cells and plasmatocytes in phagocytic reactions in the greater wax moth, *Galleria mellonella*. *Journal of Insect Physiology,* 46, 1129-1135. https://doi.org/10.1016/ S0022-1910(99)00223-1

Tsakas, S. & Marmaras, V.J. (2010). Insect immunity and its signalling: an overview. *Invertebrate Survival Journal,* 7, 228-238.

Tu, Z.L., Kobayashi, Y., Kiguchi, K., Watanabe, H. & Yamamoto, K. (2002). Effects of heavy-ion radiosurgery on the hemopoietic function of the silkworm *Bombyx mori*. *Journal of Radiation Research,* 43, 269-275. http://dx.doi.org/10.1269/jrr.43.269

Urbanski, A., Czarniewska, E., Baraniak E. & Rosinski, G. (2017). Impact of cold on the immune system of burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Insect Science,* 24, 443–454. DOI: 10.1111/1744-7917.12321

Vass, E. & Nappi, A.J. (2001). Fruit fly immunity. *BioEssays,* 51, 529-535.

Vendan E.S. (2016). Current scenario of biopesticides and ecofriendly insect pest management in India. *South Indian Journal of Biological Sciences,* 2(2), 268-271. DOI: 10.22205/sijbs/2016/v2/i2/100315

Vey, A., Matha, V. & Dumas, C. (2002). Effects of the peptide mycotoxin destruxin E on insect haemocytes and on dynamics and efficiency of the multicellular immune reaction. *Journal of Invertebrate Pathology,* 80, 177-187. https://doi.org/ 10.1016/ S0022-2011(02)00104-0

Viglianco, A.I., Novo, R., Cragnolini, C. & Nassetta, M. (2006). Actividad biológica de extractos crudos de *Larrea divaricata* Cav. y *Capparis atamisquea* Kuntze sobre *Sitophilus oryzae* (L.). *Agriscientia,* 23, 83-89.

Vlisidou, I. & Wood, W. (2015). *Drosophila* blood cells and their role in immune responses. *FEBS Journal,* 282, 1368-1382. DOI:10.1111/febs.13235

Vogelweith, F., Thiery, D., Quagliett, B., Moret, Y. & Moreau, J. (2011). Host plant variation plastically impacts different traits of the immune system of a phytophagous insect. *Functional Ecology,* 25, 1241-1247. DOI:10.2307/ 41319620

Vogelweith, F., Moret, Y., Monceau, K., Thiéry, D. & Moreau, J. (2016). The relative abundance of hemocyte types in a polyphagous moth larva depends on diet. *Journal of Insect Physiology,* 88, 33-39. http://dx.doi.org/10.1016/j.jinsphys.2016.02.010

Wago, H. (1980). Humoral factors promoting the adhesive properties of the granular cells and plasmatocytes of the silkworm, *Bombyx mori*, and their possible role in the initial cellular reactions to foreignness. *Cellular Immunology,* 54, 155-169. https://doi.org/10.1016/0008-8749(80)90198-7

Wang, Y. & Jiang, H. (2004). Prophenoloxidase (proPO) activation in *Manduca sexta*: an analysis of molecular interactions among proPO, proPO-activating proteinase-3, and a cofactor. *Insect Biochemistry and Molecular Biology,* 34, 731-742. https://doi.org/10.1016/j.ibmb. 2004.03.008

Wang, Q., Liu, Y., He, H.J., Zhao, X.F. & Wang, J.X. (2010). Immune responses of *Helicoverpa armigera* to different kinds of pathogens. *BMC Immunology,* 11(9), 12pp. https://doi.org/10.1186/1471-2172-11-9

Wheeler, R.E. (1963). Studies on the total hemocyte count and hemolymph volume in *Periplaneta americana* (L) with special reference to the last moulting cycle. *Journal of Insect Physiology,* 9, 223-235. https://doi.org/10.1016/0022-1910(63) 90074-X

Wigglesworth, V.B. (1973). Haemocytes and basement membrane formation in *Rhodnius*. *Journal of Insect Physiology,* 19, 831-844. https://doi.org/10.1016/0022-1910(73) 90155-8

Wigglesworth, V.B. (1979). Hemocytes and growth in insects. In: "Insect hemocytes"(Gupta, A.P., ed.). pp: 303-318. New York: Cambridge University Press.

Williams MJ. (2007). *Drosophila* hemopoiesis and cellular immunity. *Journal of Immunology*, 178, 4711–4716. DOI: https://doi.org/10.4049/jimmunol.178.8. 4711

Wojda, I., Kowalski, P. & Jakubowicz, T. (2009). Humoral immune response of *Galleria mellonella* larvae after infection by *Beauveria bassiana* under optimal and heat-shock conditions. *Journal of Insect Physiology,* 55, 525-531. https://doi.org/10.1016/j.jinsphys.2009.01.014

Wood, W. & Jacinto, A. (2007). *Drosophila melanogaster* embryonic haemocytes: masters of multitasking. *Nature Review: Molecular Cell Biology*, 8, 542-551.

Wu, G., Liu, Y., Ding, Y. & Yi, Y. (2016). Ultrastructural and functional characterization of circulating hemocytes from *Galleria mellonella* larva: cell types and their role in the innate immunity. *Tissue and Cell,* 48(4), 297-304. https://doi.org/10.1016/j.tice.2016. 06.007

Wyllie, A.H. (1981). Cell death: a new classification separating apoptosis from necrosis. In: "Cell Death in Biology and Pathology"(Bowen I.D. and Lockshin R.A., eds.). Chapman and Hall, London and New York 1st ed., pp: 9-34.

Xue, C.B., Luo, W.C., Chen, Q.X., Wang, Q. & Ke, L.N. (2006). Enzymatic properties of phenoloxidase from *Pieris rapae* (Lepidoptera) larvae. *Insect Science*, 13, 251–256. https://doi.org/10. 1111/j.1744-7917.2006. 00091.x

Xylander, W.E.R. (1992). Immune defence reactions of Myriapoda- A brief presentation of recent results. In: "Advances in Myriapodology" (Proceedings of the 8th International Congress of Myriapodology) (Thaler K, Meyer E, Schedl W., eds). Ber. Nat-Med. Verein Innsbruck. *Suppl.*, 10, 101-110.

Xylander, W.E.R. (2009). Hemocytes in Myriapoda (Arthropoda): a review. *Invertebrate Survival Journal*, 6, 114-124.

Yeh, S.P., Sung, G.T., Chang, C.C., Cheng, W. & Kuo, M.N. (2005). Effects of an organophosphorus insecticide, trichlorfon, on hematological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture*, 243, 383-392. doi: 10.1016/j.aquaculture.2004.10.017

Zahran, H.M. & Gad, A.A. (2013). Effect of certain plant extracts on mortality, development and haemogram of *Culex pipiens* L. mosquitos' larvae. *Alexandria Science Exchange Journal*, 34(2), 234-241.

Zaidi, Z.S. & Khan, M.A. (1975). Inverse relationship between plasmatocytes and adipohaemocytes of *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae) related to age and reproduction cycle. *Current Science*, 44, 346-347.

Zhou, Z., Mangahas, P.M. & Yu, X. (2004). The genetics of hiding the corpse: engulfment and degradation of apoptotic cells in *C. elegans* and *D. melanogaster*. *Current Topics in Developmental Biology*, 63, 91-143. https://doi.org/10.1016/ S0070-2153(04)63004-3

Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L. & Huang, Q. (2012). Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, *Spodoptera litura*. *Journal of Insect Sciences*, 12(27), 1-13. doi: 10.1673/031.012.2701.

Zhu, J.Y., Wu, G.X., Ye, G.Y. & Hu, C. (2013). Heat shock protein genes (*hsp20*, *hsp75* and *hsp90*) from *Pieris rapae*: molecular cloning and transcription in response to parasitization by *Pteromalus puparum*. *Insect Science*, 20, 183–193. https://doi.org/ 10.1111/j.1744-7917.2011. 01494.x

Zibaee, A. & Bandani, A. R. (2010a). Effects of *Artemisia annua* L. (Asteracea) on the

digestive enzymatic profiles and the cellular immune reactions of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauveria bassiana*. *Bulletin of Entomology Research.*, 100(2), 185-96. DOI: 10.1017/ S0007485309990149

Zibaee, A. & Bandani, A. R. (2010b). A study on the toxicity of the medicinal plant, *Artemisia annua* L. (Astracea) extracts the Sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). *Journal of Plant Protection Research*, 50, 48-54. DOI: 10.2478/v10045-010-0014-4

Zibaee, I., Bandani, A.R., Sendi, J.J., Talaei-Hassanlouei, R. & Kouchaki, B. (2010). Effects of *Bacillus thurengiensis* var. *kurstaki*, and medicinal plants (*Artemisia annua* L.) and (*Lavandula stoechas* L.) extracts on digestive enzymes and Lactate dehydrogenase of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). *Invertebrate Survival Journal*, 7, 251-261.

Zibaee, A., Zibaee, I. & Sendi, J.J. (2011). A juvenile hormone analog, pyriproxyfen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). *Pesticide Biochemistry and Physiology*, 100(3), 289-298. DOI: 10.1016/j.pestbp.2011.05.002

Zibaee, A., Bandani, A.R. & Malagoli, D. (2012). Methoxyfenozide and pyriproxyfen alter the cellular immune reactions of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) against *Beauveria bassiana*. *Pesticide Biochemistry and Physiology*, 102, 30-37. DOI: 10.1016/j.pestbp.2011.10.006