DISTURBED HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS OF INSECTS BY BOTANICALS AS AN EFFECTIVE APPROACH OF PEST CONTROL: A REVIEW OF RECENT PROGRESS

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To cite the article: Karem Ghoneim (2018). Disturbed haematological and immunological parameters of insects by botanicals as an effective approach of pest control: a review of recent progress, South Asian Journal of Biological Research, 1(2): 112-144.

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ARTICLE INFO
Article Type: Research
Received: 02, Nov. 2018.
Accepted: 11, Nov. 2018.
Published: 13, Nov. 2018.

ABSTRACT
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 seek for safe alternatives among which botanicals represent useful materials for the pest control. The primary objective of the present review was searching for a new control strategy of insect pests via the disruptive effects of botanicals on the haemogram parameters and the immune reactions. In this article, we discussed the effects of plant extracts and plant products on the haemogram parameters including total hemocyte population, differential hemocytes counts, histopathological deformities of hemocytes, haemolymph (blood) volume, mitotic index and heart activity. It focused, secondarily, on the innate immunity (humoral and cellular) in insects and the adverse impacts of botanicals on its mechanisms (phagocytosis, encapsulation, nodulation and melanization). As concluded in the current review, botanicals suppress the immune capability, leading to the insects become susceptible to the effects of microbes and ultimately results in the death. This can be appreciated as a new strategy for an effective control of insect pests. However, some points of future research had been provided. Also, some fieldwork should be conducted to realize the botanical impacts on haematological and immunological criteria for the pest control.

Keywords: haemogram, heartbeat, hemocyte, histopathology, immunity, phagocytosis, encapsulation, mitosis, nodulation.

List of Initials and Abbreviations
Abscisic acid (ABA), adiphohaemocytes (ADs), azadirachtin (Azt), blood volume (BV), coagulocytes (CGs), Differential haemocyte count (DHC), gibberellic acid (GA3), granulocytes (GRs), haemolymph volume (HV), indole-3-acetic acid (IAA), 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).

1. Introduction
The conventional insecticides play a vital role in the control of insect pests and the reduction of insect borne diseases for more than a half century and their use may remain necessary for some years.
Nonetheless, the intensive and discriminate uses of many conventional insecticides lead to several drastic problems, such as the environmental pollution, hazards to human and animals, like birds, fishes and mammals, 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 uses of these insecticides have result in outbreaks of secondary pests (Dubey et al., 2011) and dangerous problems of human life worldwide (Abhilash and Singh, 2009).

Therefore, many researchers and institutions are seeking for less persistent and biodegradable alternative materials to minimize these serious toxicological problems to humans and the environment and to delay 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 plant extracts and plant-derived materials as pest control agents led to what could be considered a second era of the botanical insecticides (Silva et al., 2002; Clemente et al., 2003). As reported by many 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; Bokaian et al., 2013; Hamadah et al., 2013; Adlin et al., 2015; Ben Hamouda et al., 2015; Chennaiyan et al., 2016a,b), extracts of several plant species and some plant-derived products represent an effective alternative for pest control; since they exhibit different actions against many insects, as toxicants, repellents, antifeedants, oviposition deterrents, suppressors of reproductive behavior, fecundity and fertility, growth regulators, with low pollution, quick degradation in the environment, less expensive, more effective, less toxic to natural enemies and mammals, safe for humankind and more selective in action than synthetic pesticides. Thus, the application of botanicals is considered environmentally and medically safe (Dayan et al., 2009; Lokesh et al., 2017).

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

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 (Singh et al., 2008; Pandey et al., 2010, 2012; Vogelweith et al., 2016). In last few decades, researchers are devoting a great deal of work on haemocytes and their role in insect immunity (Jones, 1959; Pandey, 2004; Pandey et al., 2010; Pandey and Tiwari, 2011; Shaurub, 2012; Ajamhassani et al., 2013; Blanco, 2016; Asiri, 2017). The primary objective of this article was the reviewing of disruptive effects of different botanicals on the
hematological parameters 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 goals in the present article was searching for new control strategies of insect pests basing on the disruptive effects of botanicals on haemogram parameters and immune reactions.

2. Total hemocyte population as influenced by botanicals

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, 1967; Arnold, 1972). Haemogram parameters include, also, haemolymph (blood) volume, mitotic index and cytological features of hemocytes. In my comprehensive article, different factors influencing the THC in insects had been discussed, such as the developmental stage, sex, age, reproductive status, circadian rhythm, temperature, moisture, habitat topography, nutrition, behavioral pattern and the measuring technique (Ghoneim, 2018). In the following sections, THC fluctuations in different insect species have been reviewed as response to many botanicals.

2.1. Increasing THC

Depending on the currently available literature, there are many reported results of increasing THC in different insects as response to botanical treatments. For examples, THC in 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 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*, initial increase of THC was recorded at early hours of treatment (Khanikor and Bora, 2012). 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 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, 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). Also, the increase of THC may be due to the activation of mitotic division of haemocytes (Ratcliffe and George, 1976) which has been activated in response to some plant extracts or plant products. Moreover, increasing THC can be considered as an immune response of an insect against 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 endocrine complex is involved in the haemocyte accumulation following some initial stimulus (Nappi, 1974). Early, Jones (1967) suggested that ecdysteroids can regulate the number of haemocytes. Because Azt could be considered as a responsible factor for the modification of haemolymph ecdysteroid titers (Redfern et al., 1982; Barnby and Klocce, 1990), some plant extracts may act as an antiecdysteroid materials promoting the THC (Ghoneim et al., 2015d).

2.2. Decreasing THC

On the other hand, decreasing THC had been reported in several insects by various plant extracts or plant-derived substances, such as the the cotton bollworm Helicoverpa armigera by oils of Artemisia annua, A. conyzoides and Azadirachta indica (Padmaja and Rao, 2000); S. litura by essential oils of Acorus calamus (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 dipped in 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 of the rice moth Corcyra cephalonica larvae on rice treated with powdered leaves of Lantana camara, Clerodendrum inerme and Citrus limon, there was a significant reduction in the THC (Morya et al., 2010). Treatment of the E. integriceps nymphs with A. annua extract led to a reduction of THC (Zibaee and Bandani, 2010a). THC of the Mediterranean flour moth Ephestia kuehniella was significantly reduced by increasing concentration of Ferula gummosa oil (Ghasemi et al., 2013b). After the treatment of 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 led to a remarkable decrease in the THC (Er and Keskin, 2016). Recently, remarkable reductions in THC in 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). After 48 hours of treatment of the 4th instar larvae of S. littoralis with LC25 of C. procerae and Atriplex halimus extracts, THC in larvae was significantly regressed (Asiri, 2017).

According to the available literature, Azt and some of its formulations considerably reduced the THC in various insect species, such as American cockroach Periplaneta americana (Qadri ans 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 last nymphal instar of the kissing bug Rhodnius prolixus led to a significant reduction in the THC. Drastic reductions 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 last instar larvae of G. mellonella, Er et al. (2017) recorded sharp reduction of THC in haemolymph at 24 and 48 h post-treatment.

To understand the THC reduction in insects by plant extracts or botanical products, it is important to point out that the hemocyte populations are influenced by the mitotic division of the circulating hemocytes (Er et al., 2010). For examples, 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 some plant botanicals (Salehzadeh et al., 2003; Huang et al., 2011). Also, the reduction of THC may result from 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; 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 supressed THC in haemolymph of S. litura larvae (Rao et al., 1984) and 5\textsuperscript{th} 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 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). 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 the 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. Contradictory results of THC in the same insect

Literature sources contain contradictory results of THC after treatment of the same insect with botanicals, 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 examples, treatment of the beetle Xanthogaleruca luteola with A. annua extracts resulted in decreased THC 6 and 12 h post-treatment but increased THC 24 and 48 h (Kohan and Sendi, 2013). Ghoneim et al. (2015d) treated the penultimate (4\textsuperscript{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 effects of other extracts depended on the concentration. In late-aged nymphs, all extracts exhibited inhibitory effects on THC. As recorded by Sadeghi et al. (2017), treatment of the 4\textsuperscript{th} instar larvae of the corn stem borer Sesamia cretica with 1000 ppm of the essential oil of Ferula ovina led to an enhancement of 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 h but an increase was observed by 24-48 h.

3. Differential haemocyte populations as affected by botanicals

In insects, there are several types of hemocytes. The most common types are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adipohemocytes (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 differing in various insect species (for some detail, see Theopold et al., 2004; Wang et al., 2010; Browne et al., 2013; Siddiqui and Al-Khalifa, 2014; Er et al., 2017).

The available literature has been enriched with many reported results about the differential hemocyte counts (DHC) alterations in various insects as response to the treatment with plant extracts or phytochemicals. For examples, feeding of 5\textsuperscript{th} instar larvae of D. chrysippus for 24 h 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 h of treatment (Pandey et al., 2007). After oral administration of sweet flag rhizome oil (A. calamus)
into 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 h of treatment. Recently, Er et al. (2017) recorded significant reduction in GRs but increase in PLs in larvae of G. mellonella only after treatment with 100 ppm Azt, whereas no differences were observed in PRs and OEs ratios. Shaurub and Sabbour (2017) investigated the impacts of acetone extract of M. azedarach fruits on the haemolymph picture of last instar larvae of A. ipsilon. They recorded increasing numbers of PLs and GRs, but decreasing numbers of PRs, SPs and OEs. Çelik et al. (2017) evaluated the effects 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 LC25 of C. procerae and A. halimus extracts (Asiri, 2017). Er and Keskin (2016) injected different concentrations of ABA into the haemocoele 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 last instar larvae of the same insect, Er et al. (2017) recorded significant increase in PLs, only at 100 ppm.

On the contrary, treatment of nymphs of E. integriceps and R. prolirus with Azt and A. annua extracts resulted in a dose-dependent decrease of PLs number (Azambuja et al., 1991; Zibae and Bandani, 2010 a, b). PLs population was declined in some insects by some plant extracts, such S. littoralis by LC25 or LC50 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. Fluctuation of hemocyte numbers in the immune challenged S. litura was found to cause a decline in PLs in haemolymph, at 24-72 h 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 48hrs of treatment with LC50 values of bark essential oil of Cinnamomum osmophloeum and the leaf essential oil of Matricaria chamomilla (Gad and El-DaKheel, 2009).

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 reduction of PLs count in haemolymph of last instar nymphs and newly emerged adult females (Ghoneim et al., 2015d). After injection of four doses of neem essential oil into the G. mellonella larvae, a significant decrease was recorded in PLs count in the haemolymph of larvae (Haszcz, 2016). Recently, addition of different doses of IAA in a diet of the A. grisella larvae resulted in the 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 in PLs count after 48 h of treatment (Sadeghi et al., 2017).

Some suggestions may be conceivable for the interpretation of PLs reduction in insects, after treatment with certain plant extracts. The decrease of PLs count in some insects after treatment with botanicals 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 may be converted into different types of haemocytes (Gupta and Sutherland, 1966). On the other hand, some plant extracts 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 sense, the transformation of PLs for cellular defence by loss of a portion of cytoplasm or by fragmentation or by gradual rounding off of fusiform PLs results in a reduction in the number of PLs (Pathak and Kulshreshtha, 1993).

In respect of 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 last instar larvae of A. ipsilon after treatment with acetone extract of M. azedarach fruits.

On the contrary, the GRs count was reduced in the fly P. surcoupî 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 manner (Azambuja et al., 1991; Zibaee and Bandani, 2010 a, b). After 48hrs of treatment of the 4th instar larvae of Cx. quinquefasciatus with LC50 values of the bark essential oil of C. osmophloeum and the leaf essential oil of M. chamomella, GRs had been remarkably reduced (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). Recently, treatment of the 4th instar larvae of S. cretica with 1000 ppm of of essential oil of F. ovina led to a reduction in GRs count (Sadeghi et al., 2017). After 48 hrs of treatment of S. littoralis 4th instar larvae with LC25 of C. procerae and A. halimus extracts, GRs count decreased in larvae (Asiri, 2017).

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 examples, 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 different concentration levels of the essential oil of F. ovina and recorded an increase of GR count at 1000 ppm but GR decrease at 2500 and 7000 ppm. Among 100, 1000 and 3000 ppm of NeemAzal, Er et al. (2017) recorded significant reduction in the number of GRs only after topical application of 100 ppm onto 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 reveal 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 increasing number of the phagocytic GRs. On the other hand, reduction in the number of GRs 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 silk worm *Bombbyx 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). Thus, decreasing of the observable GRs population may be due to the destruction of some number of these cells during this immune process against the tested botanicals.

In connection with PRs, their count had been remarkably reduced after 48h of treatment of the 4th instar larvae of *Cx. quinquefasciatus* with LC50 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 LC25 of *C. procerae* and *A. halimus* extracts, PRs count was declined in larvae (Asiri, 2017). After topical treatment of NeemAzal onto the last instar larvae of *G. mellonella*, Er et al. (2017) recorded no difference in DHC of PRs. 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 activity of hematopoietic organs responsible for their production (Pandey et al., 2012).

Considering the OEs, their count had been remarkably enhanced after 48h of treatment of *Cx. quinquefasciatus* 4th instar larvae with LC50 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 *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 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 LC25 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) recorded no difference in DHCs of OEs, regardless the concentration level. The addition of IAA in the diet of *A. grisella* larvae did not affect the OEs population (Çelik et al., 2017).

It is believed that OEs play crucial roles in phenoloxidase (PO) cascade when an immune challenge occurs (Strand, 2008). Therefore, the significant increase in their population in *Ephesia kuehniella* larvae could be led to stimulation of immune system of the plant oil-treated larvae to the secretion of PO (Ghasemi et al., 2013b). In addition, Kurihara et al. (1992) reported that the induced increase in the number of OEs supports their defensive function but 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 type. On the other hand, petroleum ether extract enhanced the CGs population in adults but tremendously prohibited it in the majority of nymphs. Thus, the predominant increasing CGs count might be attributed to their role in phagocytosis (Brehelin and Hoffmann, 1980). Shaurub and Sabbour (2017) reported that the number of SPs significantly increased in last instar larvae of *A. ipsilon* after treatment with acetone extract of *M. azedarach* fruits.

4. Histopathological effects of botanicals on the qualitative haemocyte profile in insects
Despite the botanicals represent a source of non-toxic compounds utilized for insect 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* (Quadri and Narsaiah, 1978), the bug *D. koenigii* (Saxena and Tikku, 1990), and the lepidopteran *S. litura* (Sharma et al., 2001, 2003, 2008). An earlier investigation was conducted by Shull et al. (1932) who observed granularity in the cytoplasm of haemocytes of the darkling beetle *Adesmia cancellata* after treatment with the botanicals 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 breakdown 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-0, Rizk (1991) could not observed vacuolation in the cytoplasm of PLs. Bulging of some PLs and lysis of other ones were caused by Azt in 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 h 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) found 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 vacuolation in cytoplasm and degeneration of organelles, both in PLs and GRs. After treatment of *S. littoralis* 4th instar larvae with LC50 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. In addition, some CGs had been degenerated and others appeared with destroyed membranes and extruded cytoplasmic contents, regardless the extract. The same authors observed, also, numerous vacuoles in the nuclei of some PLs by n-butanol extract and similar vacuoles appeared in 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 this plant, such as thymoquinine, 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 interfere 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 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 disruption of endocrine events (Schmutterer, 1990 a, b).

5. Other parameters of haemogram as affected by botanicals

5.1. Fluctuated blood volume

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 essential in many cases for an accurate evaluation of the THC (for detail, see Ghoneim, 2018). 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 body weight 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 obtained between the BV and THC.

5.2. Influenced mitotic index
Many authors (Tu et al., 2002; Saito and Iwabuchi, 2003; Okazaki et al., 2006) reported that the maintenance of hemocyte populations in insects is thought to be regulated by mitotic division of 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 the criterion of response to various treatments involving factors which may affect this activity (for detail, see Ghoneim, 2018).

Some botanicals promote or inhibit MI in some insects, at certain concentration levels and at certain times post-treatment. For examples, Reed and Majumdar (1998) examined the influence of Azt on the proliferation of S. frugiperda cells (SF-9). Cell kinetic studies conducted at 24 h intervals for 144 h 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 line with these findings, Azt application onto G. mellonella resulted in a decrease of the mitotic hemocytes (reduced MI), which could be possible explanation for reduced THC (Er et al., 2017). Altuntas et al. (2012) recorded increasing MI of hemocytes in G. mellonella larvae, at 5,000 ppm of GA3. On the other hand, Er and Keskin (2016) recorded 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
Heartbeat rate (the number of beats per minute) was reported to depend on different factors (for detail, see Ghoneim, 2018). Depending on the current literature, the effects of natural products on the insect heart activity are limited. Early, Orser and Brown (1951) assessed the effects of nine insecticidal products upon the rate of 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 possess wide biological activities against insects, their effects on the insect heart activity were examined only in few studies. As recorded by Ntalli et al. (2014), application of methanolic fruit extract of M. azedarach and its limonoids fraction onto the 5th instar larvae of S. exigua showed no significant effects on the heart contraction frequency in larvae but the heartbeat frequency in 1-day old pupae was remarkably decreased. Marciniak et al. (2010) 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. Application of glycoalkaloids on the continuously perfused Z. atratus heart progressively inhibited the contraction frequency; higher concentrations exerted short
and reversible cardiac arrests. In 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

Insects appear to lack the acquired immune response characteristic of the vertebrates (Strand, 2008; Berger and Jurcova, 2012). 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 defense mechanisms. The defensive physical barriers in insects include the integument and the peritrophic membrane. A single layer of cells covered by a multilayered cuticle forms the integument, 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 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 immunocyte-mediated immune responses in insects, like phagocytosis, nodulation, encapsulation and clotting (Tojo et al., 2000; Strand, 2008; Browne et al., 2013; Vlisidou and Wood, 2015). It is important to mention that the insect hemocytes have important functions on the immune system, metabolism and detoxification, and also play a crucial role in the defence of xenobiotics or microbial infection (Gupta, 1979). Together, circulating hemocytes and sessile hemocytes coordinate insect immunity against pathogen infection (Hillyer and Strand, 2014; Hillyer, 2016). Another immune defence in insects is apoptosis, which occurs generally against viral infections (Clem, 2005) and can be also induced by several environmentally 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 cytotoxic effects of chemicals (Altuntaş et al., 2012).

6.1. Insect immunocytes

Many authors (Ling and Yu, 2006; Kwon et al., 2014; Hwang et al., 2015) considered the PLs and GRs as key players in the cellular immune system, since their morphology are dramatically changed when they encounter the pathogens, as well as they have been observed engulfing and killing pathogens. The 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). Within Coleoptera, Kwon et al. (2014) revealed that GRs are specialized to perform specific functions, such as phagocytosis and
encapsulation in larvae of the beetle *Protaetia brevitarsis seulensis*. In larvae of this beetle, also, Hwang *et al.* (2015) observed that PLs occasionally engulfed bacteria and yeast. In the scarabaeid beetle *Cetonischema aeruginos*, Giulianini *et al.* (2003) observed only GRs and OEs phagocytizing the latex beads *in vivo*. 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; Izzetoğlu and Karaçali, 2010). Ling and Yu (2006) reported that PLs from *M. sexta* were the major hemocytes involved in phagocytosis of non-self-microsphere beads, whereas GRs were apparently the only hemocytes that phagocytose self-dead cells. DHCs in *G. mellonella* larvae showed that PLs and GRs were the most abundant circulating cell types in the haemolymph (Wu *et al.*, 2016).

### 6.2. Immuno-suppressive potential of botanicals on insects

The activity of the insect immune system depends on many factors, such as the population density (Reeson *et al.*, 1998), food availability (Brown *et al.*, 2009; Krams *et al.*, 2014), and environmental conditions, including temperature (Catalan *et al.*, 2012). The relationships between temperature and insect immune system functioning 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 confirmed 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 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). Sharma *et al.* (2008) recorded a suppression of such processes of GRs in *S. litura* larvae after treatment with the essential oil of *Acorus calamus* indicating 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 h, further laid emphasis on the total collapse of immunity-building mechanism of *S. litura*. Although several botanicals with 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*. 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*.

### 6.3. Major mechanisms of cellular innate immunity responses in insects

#### 6.3.1. Phagocytosis

Certain 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). 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 *Pyrhocoris apterus* adults and *S. littoralis* larvae, Berger and Jurčová (2012) conducted a particle ingestion of the NBT test on PRs, GRs, PLs and SPs. Depending on their results, phagocytic activity was on average 10% in *P. apterus*, and 50% in *S. littoralis* haemocytes. However, some of other hemocyte types may participate in phagocytosis and innate immune processes, as reviewed 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 oils of *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). Since *A. annua* extracts suppressed the phagocytosis, at different concentrations, these plant extracts may interfere with the ligand-receptor interactions that are likely to occur at the plasma membrane of specific hemocytes because the majority of interactions between cellular and humeral components of the insect immune system are receptor-mediated (Ratcliffe and Rowley, 1987).

### 6.3.2. Encapsulation

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 (Strand, 2008). Cellular encapsulation results in a multilayer cellular capsule (overlapping layers of cells) surrounding the foreign objects in insects (Götz and Boman, 1985). With regard to the contribution of hemocytes in encapsulation, GRs were reported to contact a foreign target, disintegrate or degranulate liberating material that endorses attachment of PLs. Subsequently multiple layers of PLs form the capsule. Depending on the available literature, few studies have examined the effects of plant extracts or plant products on the encapsulation in insects. Haszcz (2016) implanted nylon monofilaments (one per larva) into *G. mellonella* larvae treated with neem essential oil. Her results showed that high doses of neem oil (1.5 mg/larva) significantly decreased 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. Altuntaş et al. (2012) investigated the impacts of different doses of GA$_3$ on the apoptosis, necrosis, encapsulation and melanization responses in *G. mellonella* larvae. Encapsulation rates of larval hemocytes were dependent on the extent of encapsulation and time but not treatment groups.

### 6.3.3. Nodulation

Nodulation is a cellular immune process in insects whereby hemocytes recognize a foreign material and insulate it within the haemocoel as well as aggregate large numbers of invading bacteria (Mullen and Goldsworthy, 2003; Marmaras and Lampropoulou, 2009). 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 microorganisms (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, little information exists in the available literature concerning the affected nodulation in insects by plant products. Azambuja *et al*. (1991) recorded an inhibited nodulation
response after Azt treatment to last nymphal instars of *R. prolixus*. A year later, Azambuja and Garcia (1992) administrated Azt, via a blood meal, to last nymphal instar of *R. prolixus* and recorded a reduction of immune reactivity, as indicated by a significant reduction in 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 last instar larvae of *G. mellonella*, Er et al. (2017) recorded a dose-dependent decrease in the level of nodule formation.

6.4. Melanization as a humoral defense in insects

As previously reviewed, the complex enzymatic cascade that regulates coagulation or melanization of haemolymph (also called humoral encapsulation) is one of the humoral immune defenses in insects. Moreover, Castillo et al. (2011) reported that 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 a crucial role in the innate immune responses of insects, which catalyzes the biosynthesis of quinones and other reactive intermediates to eliminate invading pathogens and parasites. Most reports indicated that proPO is synthesized predominantly by hemocytes, especially in GRs and OEs (Williams, 2007).

With regard to the effects of botanicals 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 indicate ability of the essential oils to induce the immune response in larvae (Khanikor and Bora, 2012). Koodalingam et al. (2013) examined 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*. An initial delay in the visible cuticular melanization reaction was recorded in the larvae, thereby indicating a possible impact of *S. emarginatus* kernel extract on the PO system of the *A. aegypti* larvae. Huron et al. (2016) treated the adult females of the aphid *Aphis carricivora* with LC50 of acetone extracts of Lupine *Lupinus termis* and lemon grass *Cymbopogon citratus*. These authors recorded an activation of POs, as well as the enzyme activity was higher in aphids treated with *C. citratus* than that treated with *L. termis*. Recently, 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 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 injection of Azt into the haemocoel of the locust *S. gregaria*, the production of immune proteins was induced (Annadurai and Rembold, 1993). Also, Ayaad et al. (2001) injected Azt into haemocoel of the late larval instars of the flesh fly *P. surcoufii* and recorded an induction of the production of immune proteins and a significant suppression of PO activity of haemolymph even when the activators laminarin, D-chymotrypsin and methanol were present. The impact of GA3 on the melanization response of *G. mellonella* larvae had been investigated by Altuntaş et al. (2012). The extent of melanization of hemocytes exhibited a difference related to time, since a decrease of melanization was observed at 24 h-treated larvae, suggesting the negative effect of GA3 on the cell immune responses in *G. mellonella* larvae.
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. feeding, moulting, reproduction and immune system (Pandey et al., 2012). It is important to mention that the insect hemocytes have important functions in metabolism and detoxification, and also play a crucial 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, cytological features of hemocytes and heart activity, serves as a very good indicator of the insect physiology and the environmental adaptability in each developmental stage of insects (Sharma et al., 2008; Ghasemi et al., 2013a, b; Bardoloi et al., 2016). Also, the insect haemogram is suggested to be a useful tool for investigation of 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 of the pest control by plant extracts and botanical products through their disruptive effects on the haematological parameters and immune reactions of insect pests.

As previously reviewed, different plant extracts and botanical products are found to exhibit impairing effects on the insect haemogram parameters. For examples, the altered THC and the structural abnormalities of hemocytes in insects are frequently used to demonstrate the cytogenetic damage caused by phytochemicals (Yeh et al., 2005; Altuntaş et al., 2012; Pandey et al., 2012). The results of some studies revealed that different plant extracts cause much variation in the proportions of hemocytes in various insects (Er et al., 2017; Shaurub and Sabbour, 2017). In addition, the cytopathological effects of plant products on the hemocytes have been reported in a few insect species (Sharma et al., 2001, 2003, 2008). 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). Also, the influence of botanicals on the insect heart activity was evaluated in few studies (Marciniak et al., 2010; Ntalli et al., 2014).

With regard to the immune reactions in insects and their disruption by some plant extracts and botanical products, many authors (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 on the suppression of immune capability in insects. For example, THC in haemolymph reflects the immune ability for dealing with pathogens or chemicals (Asiri, 2017) since the reduction in THC after treatment with the 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 substances suppress the immune capability, leading to the insects become susceptible to the effects of microbes (Zibae et al., 2012) and ultimately results in the death. By compromising the insect innate immunity, one can develop a new strategy for an effective control of insect pests (for reviews, see James and Xu, 2012; Haszcz, 2016; Liu et al., 2017).

8. Summary points
The majority of different reviewed aspects, in the present article, 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 as response to the botanical treatment. A number of scenarios had been provided to interpret this THC increases. On the contrary, decreasing THC had been reported in several insects by various plant extracts or plant-derived substances. Also, different reasons of THC decreasing were suggested.
* In many insects, DHCs altered as 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 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. 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 may be other hemocytes) of larvae and pupae of some insects had been suppressed as 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 form the capsule containing a foreign target. Few studies have examined the effects of plant extracts on the 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 challenge with the 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. Effects of some botanicals on the melanization of insect hemocytes can be explained by their effects on PO activity.

* 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 the death. This can be appreciated as a new strategy for an effective control approach of the insect pests.

9. Conclusions and future research needs

As shown the present review, different plant extracts and plant products are found to exhibit impairing effects on the insect haemogram parameters, such as alteration of THC and DHCs as well as cause detrimentally structural abnormalities of hemocytes and reduction of hemocyte mitosis. Also, the influence of botanicals on the insect heart activity was reported. The influenced 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 results in the 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 future, such as isolation of the active ingredients in plant extracts, antimitotic activities of botanicals and their adverse effects on 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 field work should be conducted to realize the botanical potential for recommendation of this approach of pest control.

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