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DIFFERENCE IN CUTICLE COMPONENT AND IMMUNOCOMPETENCE IN NURSE AND FORAGER WORKER HONEYBEE (*APIS MELLIFERA L)*

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ABSTRACT

The aim of this work is to study the difference of physiology between the worker bee nurse and forager (*Apis mellifera intermissa*). The chosen physiological characteristics were the component of the cuticle (protein-chitin content) and the measure of the efficiency of immune system (the total number of haemocytes (THC), the normal haemocytes and the relative mass of fat body). The THC is widely used as an indicator of cellular immunocompetence of insects. The normal haemocytes, also referred to immunocytes, indicate the integrity of cellular immune system. The fat body is an indirect measurement of induced humoral immunocompetence. The THC and the normal haemocytes were determined by the method described by Amdam *et al.,* (2004). For the estimation of the cuticular abdominal protein-chitin content, the method described by Berghiche *et al.,* (2007) was employed. The relative mass of fat body was determined using an ether extraction method according to Doums *et al*., (2002) and Wilson-Rich *et a*l., (2008).

The results show that a considerable percentage of a cuticular protein and a decrease of chitin was observed in nurse compared to forager. The older bees exhibited a strong reduction in the immun parameters.

Keywords: *Apis mellifera intermissa, cuticle component, immunocompetence*.

INTRODUCTION

Honeybee, like insects, are known to possess physiological defenses to combat pathogens. The primary defense include the cuticle as the first line of defense and the effectif immune system resolved into broad categories: cellular and humoral components (Jiravanichpaisal *et al*., 2006). The arthropod cuticle is a remarkable and versatile biological material commonly composed of chitin (polymer of N.

Acetyl glucosamine) and proteins (Cribb *et al.,* 2010). The cellular components of insect immunity, named heamocytes, are able to phagocyte, nodulate and encapsulate (Lavine and Strand, 2002; Jiravanichpaisal *et al*., 2006). The total number of haemocytes (THC) is widely used as an indicator of cellular immunocompetence of insects. The normal haemocytes, also referred to immunocytes, indicate the integrity of cellular immune system (Amdam *et al.,* 2005). The humoral reactions involve induced synthesis of antibacterial proteins (by fat body and haemocytes), coagulation and melanisation (Hoffmann *et al.*, 1996; Lavine and Strand, 2002; Lemaitre and Hoffmann, 2007), which is catalysed by the (propheno-) phenoloxidaese (PO). This PO-mediated melanin synthesis plays a major role in an insect's immune defense and in cuticular sclerotisation and quinone production (Lavine and Strand, 2002). According to Andersen (2010), the quinines react with cuticular proteins stabilizing cuticle structure.

Honey bees, the important social insects, exhibit age division of labor, a consequence of individual bees changing jobs as they grew older. The division of labor and the transition of the nurse bees to perform foraging tasks were suggested to be impacted by changes in brain chemistry, brain structure, endocrine activity and gene expression (Robinson *et al*., 1987, 1989; Huang *et al*., 1994; Huang and Robinson,1996; Lass and Crailsheim, 1996; Ben-Shahar *et al.,* 2000; Schulz and Robinson, 2001; Robinson, 2002; Remolina *et al.,* 2007; Heylen *et al*., 2008; Liu *et al.,* 2011; Greenberg *et al*., 2012). The changes physiological functions have been extensively studied in worker honeybees through the ages. In recents years, significant interests have been shown in studying honeybee humoral, cellular immune response (Bedick *et al*., 2001; Amdam *et al.,* 2004, 2005; Klaudiny *et al.,* 2004; Lourenço *et al.,* 2005; Yang and Cox-Foster, 2005; Evans, 2006; Wilson-Rich *et al.,* 2008; Alaux *et al.,* 2010) and characteristcs of integument (Thompson and Hepburn, 1978; Nemtsev *et al*., 2001; Elias-Neto *et al.,2009,* 2014; Seehuus *et al.* 2013; Kaya *et al.*, 2015). We have a few information about the component of cuticle (Thompson and Hepburn, 1978) and cellular immune component of honeybee age-related division labor (Amdam *et al.,* 2005; Schmid *et al*., 2008). Thompson and Hepburn (1978) used abdominal tergites for determination the chemical and mechanical properties of the pharate adult honeybees cuticle. To our knowledge, the report is the first work, which study the cuticle component using the abdominal integument (tergite and sternite) of two ages (nurse and forager). Seehuus *et al.,* (2013) studied the genes encoding cuticle proteins in nurse and forager. A little work is known about another measure of humoral immune defense, the relative percentage of fat body in the abdomen (Wilson-Rich *et al.,* 2008).

The aim of this study is to investigate the difference of the cuticle component and the immunocompetence between the nurse and forager honeybee (*Apis mellifera intermissa*). The chosen physiological parameters were the cuticular abdominal protein-chitin content, the total number of haemocytes (THC), the normal haemocytes and the relative percentage of fat body.

MATERIAL AND METHODS

The experiments were carried out in an apiary of honeybees derived from *Apis mellifera intermissa* during the summer 2015 in northern Algeria (Isser 36° 43' N., 3 ° 40' E). Bees were determined to be foragers if they returned with pollen loads in their corbicular or had a distended abdomen (signifying nectar or, less likely, water foraging) (Huang and Robinson, 1996). The nurses were collected when they entered into the cells and were nursing the larvae (Liu *et al*., 2011). For the estimation of the cuticular abdominal protein-chitin content, the head and thorax of nurse and forager were removed as well as all body appendages before being dissected. The abdomen was carefully cleaned from all adhering tissues. The abdominal integument were washed for 24 h in ether-chloroform (1:1, v/v) and dried to constant weight at 60 °C. Chitin and total soluble protein were assessed in the cuticle of nurse and foragers according to the method described by Berghiche *et al., (*2007). The total number of haemocytes (THC) was conducted by the method described by Amdam *et al.,* (2004). The cells counts using light microscope were done 5–10 min after filling the neubauer hemocytometer. The haemolymph was drawn with a calibrated microcapillary inserted dorsally into the bee between the second and third abdominal tergite of honeybee worker. Clear and slightly yellow hemolymph was drawn out by capillary action. The normal haemocytes count was determined by the method described by Amdam *et al.,* (2004). The fat body mass was estimated using an ether extraction method according to Doums *et al.,* (2002) and Wilson-Rich *et a*l. (2008). The percentage values are given as dry mass of fat body / dry mass of the abdomen.

Results are expressed as means ± standard deviation (s). The number of honeybee tested per series is given with the results. The significance between the 2 group (nurses and foragers) was estimated using Student's t test at 5% level.

RESULTS AND DISCUSSION

This study examined the abdominal cuticle components (protein-chitin) and an effectif immune parameters of the two ages of worker honeybee, nurse (young bees) which do job in the hive such feeding larva and forager (oldest bees) which perform tasks in a colony forage for nectar and pollen outside the hive. The effective cellular and humoral immune include respectively the measures of the total haemocyte count (THC), the normal haemocytes (immunocytes) and the relative mass of fat body. The results of the component of cuticle, the first line of defense and immunocompetence parameters of nurse and forager *(Apis mellifera intermissa)* are presented in the Fig 1.

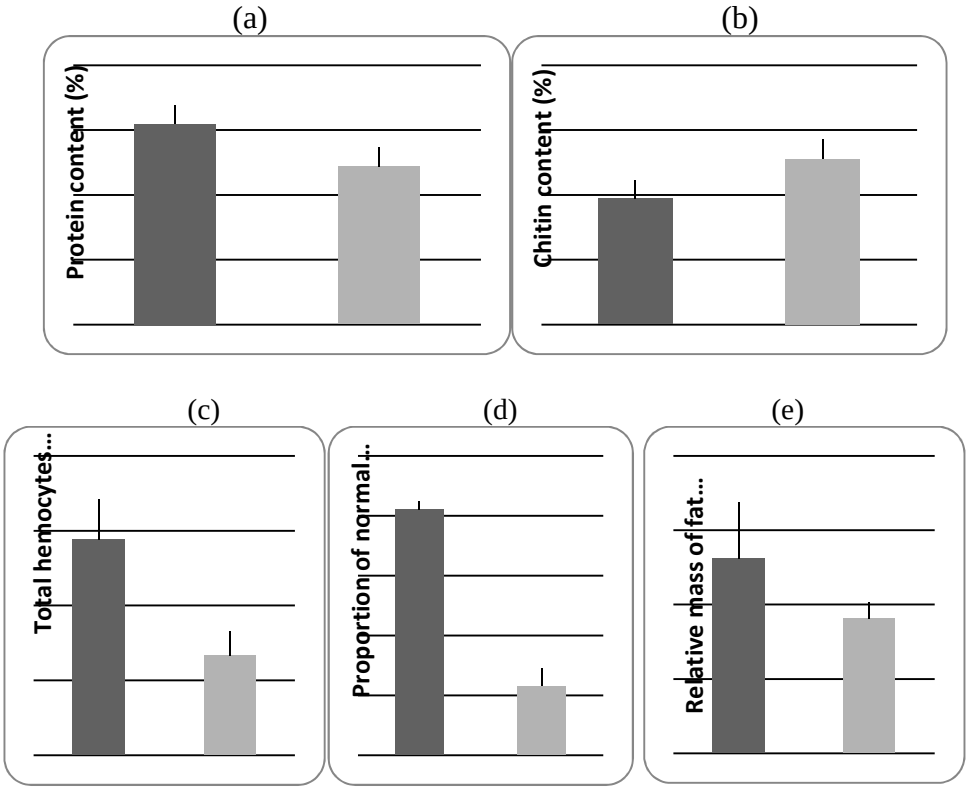

Figure 1. Components of abdominal cuticle and the measures of immunocompetence parameters of nurse and forager bees (*Apis mellifera intermissa*). a and b: Protein-chitin content respectively (n=13), c: Total haemocyte count (n=10), d: Normal haemocytes (n=4), e: Relative mass of fat body (n=20).

The exoskeleton of cuticle of arthropods is an important feature that contributes to their great success in colonizing numerous habitats on earth (Moret and Moreau, 2012). The cuticle is an important assembly of chitin and proteins (Charles, 2010). In the honeybees, ontogenetically, two basic types of exoskeleton can be identified in the honey bees; the flexible and colorless (unpigmented) larval and pupal exoskeletons and the rigid (highly sclerotized) and pigmented adult exoskeleton (Elias-Neto *et al*. 2014). According to Vincent *et al*., (2004**),** the protein is responsible for the stiffness and elasticity of the cuticle. In general, the soft cuticle has a much content of chitin (50% dry weight) than the stiff cuticles (15-30% dry weight). According to Willis (1999), the physical properties of insect cuticle depend on several things, cuticular lipids, the proportion of chitin, the nature and quantity of sclerotizing agents and the sequence of the constituents proteins. Charles (2010) suggested that the physical properties are determined largely by the proteins it contains and vary widely with developmental stages and body regions. The corpses of dry dead bees, gathered after wintering, contained 50-80% protein,

10-20% chitin, 20-30% melanin and 2-3% mineral substances but the dead bees after CO_2 extraction contained the 45-50% protein, 20-22% chitin, 20-22% melanin and 2-3% mineral substances (Nemtsev *et al.,* (2001).

Our results show that the mean protein content in the abdominal cuticle of nurse and forager (*Apis mellifera intermissa)* was 61.83 ± 5.69 % and 48.8 ± 6.1 % respectively (Fig 1a). The chitin level of nurse and forager was respectively 38.91 ± 5.59 % and 51.18± 6.11% (Fig 1b). Thompson and Hepburn (1978) noted that the percentage of abdominal chitin for the honeybee pharat adult was found to be around 19.3 %. However, the chitin content of whole body of honeybee as about 19% (Nemtsev *et al*., 2004). Kaya *et al.,* (2015) found a differences amoung the body parts of honeybee, legs (13.25%), thorax (6.79%), 8.9% for head, 8.61% for abdomen and 7.64% for wings.

Cuticular protein-chitin content from others insects varied with species and their developmental stages. The percentage of protein in the cuticle of the fifth instar nymphs of *Schistocerca gregaria* and the pupal integument of *Tenebrio molitor* is around 70% respectively (Berghiche *et al.,* 2007; Tail *et al.,* 2015). The dry weight chitin contents of the adult *Colorado potato* beetle and larvae were determined as 20% and 7% respectively (Kaya *et al.,* 2014). The chitin content from *Holotrichia parallela* is around 15% (Liu *et al*., 2012). Kaya *et al.,* (2015) found that the level of these component of *Vespa crabro, Vespa orientalis* and *Vespa germanica* were respectively 8.3%, 6.4% and 11.9%.

As is known, physicochemical properties of cuticle are highly affected by extraction method (Thomson *et al.* 2004). The exoskeleton (cuticle) of insects varies widely in shape, biochemical properties and fuctions, which are inherent to the biological species, developmental stage, besides showing, wealth of architectural specialization and nuances in the different body regions (Elias-Neto *et al.,* 2014).

From our results, two main differences were observed. First, we noted that the content of protein was significantly greater in nurse than in forager ($P < 0,0001$). The second, the percentage of chitin content of nurse was significantly lower than in forager ($P < 0,0001$). This is in agreement with previous results showing that nurses have a high investment in cuticular protein, chitinase and chitin metabolic process with 36 expressed genes in comparison to foragers. The older bees also possessed a lowest number of expressed genes in lipid metabolism and a higher number in carbohydrate metabolism (Seehuus *et al.,* 2013). The same authors reported that the difference between foragers and nurses in cuticular component correlate with changes in proteosynthetic activity of fat body. The tissue, a functional homologue of the mammalian liver (Lemaitre and Hoffmann, 2007), is the main site of energy, antimicrobial peptides, protein storage (Lemaitre and Hoffmann, 2007; Roma *et al.,* 2010). It is also one of the most important tissues of maintenance and reproduction process (Roma *et al.,* 2010).

Our results show that nurse bees had greater fat body (52.32 ± 15.13 %) mass than foragers (36.36 ± 4.37 %)(Fig 1e). Our observations are consistent with Wilson-Rich *et al*., (2008)**.** The fat body forager was used as a source of energy involved in stress response, behavior, sensory, learning and memory (Seehuus *et al.,* 2013). It known that the somatic maintenance machinery in forager is a physiologically expensive option cost (Alaux *et al*., 2010; Seehuus *et al.,* 2013). In order to save energy costs, the plausible strategy for the forager, the superorganisme, was also to abandon hemocytic immunity (Seehuus *et al.,* 2013).

As seen in figure 1, the young bees show that the mean of the THC (14 420 ± 2718.2 cells / µl) was significantly higher than in forager (6 660 ± 1644.7 cells / µl) (Fig 1c). The similary observation was found by Amdam *et al.,* (2005); Schmid *et al.,* (2008); Wilson-Rich *et al.,* (2008). As for THC, the nurse possess a higher number of the proportion of immunocytes than forager ($p<0,0001$)(Fig 1d). The similar disruptions of host immune functions have been reported by Amdam *et al.,* 2005; Schmid *et al.,* 2008). Amdam *et al*., (2005) suggested that the juvenile hormone level, which accompanies onset of foraging behavior, induces extensive haemocyte death through nuclear pycnosis. According to Schmid *et al.,* (2008), this loss of immune competence has been regarded advantageous with respect to an already high mortality rate due to foraging and to redistribution of energy costs at the colony level**.** The older worker bees were still significantly less resistant to the three physiological stressors (starvation, thermal and oxidative stress) than the younger bees. Khater *et al.,* (2011) in Abou-Shaara (2014), the forager bees have different n-alkane profiles than the nurse bees with a higher quantity of n-alkane which may help the forager bees to tolerate the ambient conditions. Remolina *et al*., (2007) reported that the forager would be more susceptible to direct attacks from predators and benefit from more protection in the form of stronger cuticle.

CONCLUSIONS

Several remarkable features distinguish nurse, which do job inside the hive from forager, which leave the colony for foraging activity. Pronounced differences between the two ages were found in the abdominal cuticular content of protein-chitin. The forager exhibited a strong reduction of the THC and the number of functional haemocytes. The relative mass of fat body decrease dramatically in older bees compared with the young bees. The differences are due to intrinsic physiological differences, the differential exposure to extrinsic factors such as predation, thermal stress and physical exhaustion. Possessing the strongest cuticle would be beneficial to forager lacking effective escape responses.

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