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Osmotic concentration in three races of honey bee, *Apis mellifera* L. under environmental conditions of arid zone

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ABSTRACT

Hemolymph osmolarity has great effect on honey bee health, especially in arid and semi-arid zones. It regulates water and nutrients in stressed tissues. Osmotic concentration in three races (*Apis mellifera ligustica*, *A. m. carnica* and *A. m. jemenitica*) of *Apis mellifera* was tested in central Saudi Arabia during spring and summer seasons in 2015. Newly emerged bee workers were first marked and later their hemolymph was extracted after intervals of 1, 5, 10, 15, 20 and 25 days. A significant positive correlation between age and osmolarity was found in all three races during spring and summer seasons. The lowest combined osmotic concentration for all three races was found after 1 day interval, while the highest osmotic concentration was recorded after 25 days. Among all races, *A. m. ligustica* showed significantly high osmotic concentration after 25 days in spring and summer seasons as compared to the other two races. Only *A. m. jemenitica* showed similar osmotic concentration after 10 and 15 days in both spring and summer seasons compared to other two races. Mean osmotic concentration of all three races was significantly different after 20 and 25 days in spring and summer seasons. Overall mean recorded during summer was significantly higher than the mean of spring season. Combined osmotic concentration in young drones of all races was significantly lower than that of old drones during spring and summer seasons.

1. Introduction

The honey bee (Apidae: Hymenoptera) produce valuable agricultural products such as honey, pollen, and wax. It also provides pollination services to numerous crops (Wakhal and Bhujbal Pais., 1999). Honey bee colony generally consists of a reproductive queen, number of drones depending on time of the year, and thousands of worker bees. Honey bees are faced with several natural and artificial stressors. Natural stressors include extremes of weather factors (temperature, rainfall, drought, etc), natural disasters, predators, parasites, and diseases (Owens, 1971).

Under stress conditions, it is critical for honey bees to maintain water balance, changes of which can alter hemolymph osmolarity. Alteration in osmolarity has important impacts on health of honey bees. Hemolymph of the insect acts as a source of water and nutrients for stressed tissues (Atmowidjojo et al., 1999). An increase in hemolymph osmolarity triggers changes in transport rates through the proventriculus (Crailsheim, 1988).

Several studies have revealed that osmolarity varies across honey bee species, different maintenance conditions, and environmental conditions. Leonhard and Crailsheim (1999) reported that osmolarity in drones of *A. m. carnica* ranged from 334 to 532 mOsm/L. The level was low at emergence, increased up to 3 days, and then was constant until the 16th day. The highest osmolarity of 532 ± 38 mOsm/L was recorded in 25 days old drones. Previous studies reported 573 mOsm/L osmolarity in honey bee workers (Crailsheim, 1985).

Wild and domestic honey bees also differ in hemolymph osmolality. Hemolymph osmotic pressure of domestic honey bees was higher than that of wild honey bees (Atmowidjojo et al., 1999). Osmolarity of insect hemolymph increases during dehydration. However, some species can sustain stable osmolarity even in severe dehydration periods (Cohen, 1984, & Riddle, 1986).

Honey bees are very important for agriculture in Saudi Arabia. In central Saudi Arabia, environmental conditions are character-

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ized by high temperature and low humidity. Climate in this region is classified as the *BWh* type (*BW* = desert climate with accumulated annual precipitation less than 50% of the potential evapotranspiration, and *h* = hot steppe or desert) according to the Köppen-Geiger climate classification (Kottek et al., 2006; Rubel and Kottek, 2010).

Beekeeping is greatly challenged by these kinds of environmental conditions. The indigenous honey bee *A. m. jementica* was found to perform well under harsh environmental conditions as compared to imported races i.e. *A. m. ligustica and A. m. carnica.* Further, the *A. m. jemenetica* showed significantly lower water loss as compared to exotic races (Alqarni, 2006; Alqarni et al., 2014).

Abou-Shaara et al. (2012) reported that *A. m. jemenitica* was more heat tolerant than the imported race, *A. m. carnica*. In a recent study, Alattal and AlGhamdi (2015) compared colony losses in three races due to heat and found high colony losses in exotic races, *A. m. carnica* and *A. m. ligustica*, as compared to *A. m. jemenitica*.

Beekeeping in central Saudi Arabia is focused on the presence of two main wild honey plants (*Acacia gerrardii* and *Ziziphus* spp.) that are found in several valleys and oases. Both plants flowering seasons extend through out summer (mid June-late September).

Because of the importance of honey bees, especially the native race *A. m. jemenetica*, to agriculture in Saudi Arabia, it is important to understand how temperature and humidity affect hemolymph osmolarity in different honey bee races typically used in apiculture. Therefore, this experiment was carried out to explore the effect of stress conditions on osmolarity in honey bee workers of different ages, young and adult drones in indigenous and exotic honey bee races.

2. Material and methods

Bee colonies were kept at the apiary of the bee research unit at Dyrab Agriculture Research Station (24.409551, 46.657564) 40 km south of Riyadh. Laboratory experiments were carried out at the Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.

2.1. Honey bee races

Three *Apis mellifera* races were used for the experiments. Colonies of the native Saudi honey bees *Apis mellifera jemenitica* were obtained from a reliable source in southern Saudi Arabia, while *Apis mellifera carnica* and *Apis mellifera ligustica* queens were imported from certified queen producers from Egypt and Jordan, respectively.

Imported queens were introduced to five frame boxes and were left until they start egg laying, and the colony population was subsequently and regularly confirmed to be the offspring of the introduced queens by morphometric characteristics. The time period for acclimatization of imported queens was up to 60 days. All colonies were treated equally following normal apicultural practices; i.e. feeding on 1:1 sugar syrup, inspection, and beside no chemicals were used. Three equal strength colonies were randomly selected, one colony for each race.

2.2. Collection and sample preparation of hemolymph

Soon after emergence the honey bee workers were marked in the colonies on their thoraxes while on the comb using paint markers of Uni Paint® (yellow, green, and white). Approximately 90–100 bees were marked. Bees were marked in order to know their exact ages. Marked bees were collected after intervals of 1, 5, 10, 15, 20, and 25 days.

For hemolymph extraction, a total of 10 honey bee workers of each age were collected from each colony (race). Also, 10 drones (five young drones from inside colonies and five adult drones on orientation flights) were also collected from each of the three races. The experiment was performed during spring and summer seasons. Osmolarity was measured following the method of Mayack and Naug (2010).

After collection, the honey bee workers as well as drones were killed by freezing. After killing, their wings were removed and mouth parts were glued to avoid any possible contamination from the gut. The distal parts of the antennae were cut and removed. Later on, bees were put upside down in a centrifuging tube and spun at 16,000 Relative Centrifugal Force (RCF) for 30 s. The hemolymph flowed from the cut ends of the antennae. A volume of 1 µl of sample was collected and diluted in distilled water at ratio of 1:10, and then vortexed thoroughly. The whole process was conducted on ice to avoid degradation of the hemolymph.

2.3. Measurement of osmolarity

Osmolarity or osmotic concentration is the number of osmoles of a solute per liter of solution. It is expressed as mOsml/L or Osm/L (Erstad, 2003). Osmolarity in the hemolymph was determined by an osmometer (Wescor-5520, Germany). A volume of 10 µl sample was used, kept on ice during measurement, and displayed readings were recorded.

2.4. Statistical analysis

The recoded data were subjected to statistical analysis. Data were examined for normality and homogeneity using Kolmogorov–Smirnov and Levene tests, respectively. The means were tested using ANOVA, and subsequently separated using Duncan's Test at $p \leqslant 0.05$.

3. Results

The effect of age on the osmotic concentration (osmolarity) in three *Apis mellifera* races during spring and summer seasons of central Saudi Arabia is presented in Tables 1 and 2. In spring (Table 1), a positive correlation between age and osmotic concentration was found in all three races. In *A. m. ligustica* the osmotic concentration differed significantly from the previous day in all recorded days. Minimum osmotic concentration (366.4 ± 22.4 mOsm/L) was recorded after emergence and increased significantly after 5, 10, 15, 20 and 25 days, whereas maximum osmotic concentration (540.2 ± 38.1 mOsm/L) was recorded after 25 days. In *A. m. carnica* significant differences were observed at 1, 5, 10, and 15 days after emergence. However, there was no significant difference between 20 and 25 days. In *A. m.*

Table 1
Osmotic concentration (Osmolarity) mOsm/L of three *Apis mellifera* races (*A. m. carniac, A. m. ligustica, and A. m. jemenitica*) during spring 2015 in Riyadh, central Saudi Arabia.

Workers age (n = 10)	Osmolarity mOsm/L (Mean ± S.D)		
	A. m. ligustica	*A. m. carnica*	*A. m. jemenitica*
1 day	366.41 ± 22.44Ea	341.15 ± 39.28 Da	336.4 ± 28.77 Da
5 days	419.12 ± 28.21 Da	421.81 ± 32.92Ca	414.11 ± 19.8Ca
10 days	420.71 ± 37.21 Da	434.52 ± 37.18Ca	445.43 ± 35.48Ba
15 days	451.42 ± 31.36Ca	472.61 ± 41.33Ba	451.81 ± 35.73Ba
20 days	525.33 ± 29.35Ba	515.62 ± 48.84Aa	494.22 ± 42.13Aa
25 days	540.23 ± 38.17Aa	500.81 ± 33.95Ab	493.52 ± 38.05Ab

*Means followed by the same letter are non-significant at 5% ($p \leqslant 0.05$) level of probability (days = A–E, races = ab).

Table 2
Osmotic concentration (Osmolarity) mOsm/L of three *Apis mellifera* races (*A. m. carniac*, *A. m. ligustica*, *and A. m. jemenitica*) during summer 2015 in Riyadh, central Saudi Arabia.

Workers age (n = 10)	Osmolarity mOsm/L (Mean ± S.D)		
	A. m. ligustica	*A. m. carnica*	*A. m. jemenitica*
1 day	362.40 ± 29.61Fa	338.91 ± 27.67Eb	333.40 ± 9.44 Eb
5 days	403.30 ± 26.46Ea	407.71 ± 16.14 Da	408.41 ± 18.68 Da
10 days	430.11 ± 31.37 Da	428.90 ± 40.75Ca	444.32 ± 22.99Ca
15 days	464.81 ± 22.78Ca	466.50 ± 26.07Ba	452.60 ± 22.37Ca
20 days	538.50 ± 47.24Ba	529.90 ± 41.44Aa	514.11 ± 47.12Ba
25 days	557.31 ± 38.41 Aa	531.80 ± 46.17Ab	535.90 ± 48.64Ab

*Means followed by the same letter are non-significant at 5% ($p \leqslant 0.05$) level of probability (days = A–F, races = ab).

jemenitica, the minimum osmotic concentration (336.3 ± 28.7 mOsm/L) was found after 1 day, and it increased across days to a peak after 20–25 days. Across races, significant differences were recorded at 25 days. *A. m. ligustica* showed significantly higher osmotic concentration (540.23 ± 38.17 mOsm/L) than *A. m. carnica* (500.81 ± 33.95 mOsm/L) and *A. m. jemenitca* (493.52 ± 38.05 mOsm/L).

The osmotic concentration of the three *A. mellifera* races in summer is presented in Table 2. As in spring, a significant, positive correlation was also found between age and osmotic concentration. In *A. m. ligustica* significant differences were found among all recorded days. The minimum osmotic concentration (362.4 ± 29.6 mOsm/L) was recorded at 1 day after emergence, while the maximum (557.3 ± 37.9 mOsm/L) occurred after 25 days. Osmotic concentration of *A. m. carnica* increased significantly at each recording until 20 days after emergence. There was no significant difference between osmotic concentration at 20 and 25 days of emergence. *A. m. jemenitica* also showed significant increase in hemolymph osmotic concentration on 1, 5, 10, 20 and 25 days. However, there was no significant difference between osmotic concentrations at 10 and 15 days. Significant differences were found between the three races only after 1 and 25 days of emergence. Similar to spring, *A. mellifera ligustica* showed significantly higher osmotic concentration (557.31 ± 38.41 mOsm/L) as compared to *A. mellifera carnica* (531.80 ± 46.17 mOsm/L) and *A. mellifera jemenitca* (535.90 ± 48.64 mOsm/L) (Table 2).

The overall osmotic concentration of the three *Apis mellifera* races during spring and summer is presented in Fig.1. Values between the two seasons' osmolarity were not different significantly up to 15 days after emergence. Significant differences were found only at 20 and 25 days, with the summer season showing significantly higher osmolarity values than the spring. This was reflected on the total percentage of increase in osmotic concentration through 1–25 days after emergence that showed 41.5% and 48.3% in spring and summer respectively (Table 3).

Data in Table 4 shows that all three races have significantly different osmotic concentration between young and old drones in spring season. Values for young drones were 394.51 ± 24.09 mOsm/L, 398 ± 25.84 mOsm/L and 389.66 ± 34.18 mOsm/L for *A. m. ligustica*, *A. m. carnica* and *A. m. jeminitica* respectively, whereas old drones values were 434.33 ± 23.99 mOsm/L for *A. m. ligustica*, 425.17 ± 19.12 mOsm/L for *A. m. carnica* and 412.17 ± 23.70 mOsm/L for *A. m. jemenitica*. Combined osmotic concentration of young and old drones of each race was not significantly different during spring season.

Table 5 shows the hemolymph osmotic concentration data of young and old drones during summer season. Young drones showed significantly lower osmotic concentration than old drones in all three races. In old drones the osmotic concentration was 507.1 ± 20.9, 497.6 ± 8.1 and 493.5 ± 5.2 mOsm/L in *A. m. ligustica*, *A. m. carnica* and *A. m. jemenitica* respectively. In summer, significant difference was recorded. *A. m. ligustica* drones showed significantly higher osmotic concentration than *A. m. jemenitca* and *A. m. carnica* (Table 5).

4. Discussion

The present findings are in line with those of Leonhard and Crailsheim (1999) who reported that honey bee osmolarity increased with age. We reported that in one-day old bees, osmolarity ranged between 334 and 370 mOsm/L, and afterward increased significantly to 400 mOsm/L after 5–10 days. In both spring and summer seasons, significant differences were found between osmotic concentration of the two races *A. m. ligustica* and *A. m. carnica* after 10 and 15 days. Only *A. m. jemenitica* showed no significant differences in osmotic concentration of the same time periods. This difference implies that native honey bees (*A. m. jemenitica*) are possibly less sensitive to heat stress as compared to exotic honey bees and better adapted physiologically to the local environment. Alqarni (2006) reported that during summer season the native bee race forging activity was significantly high as compared to

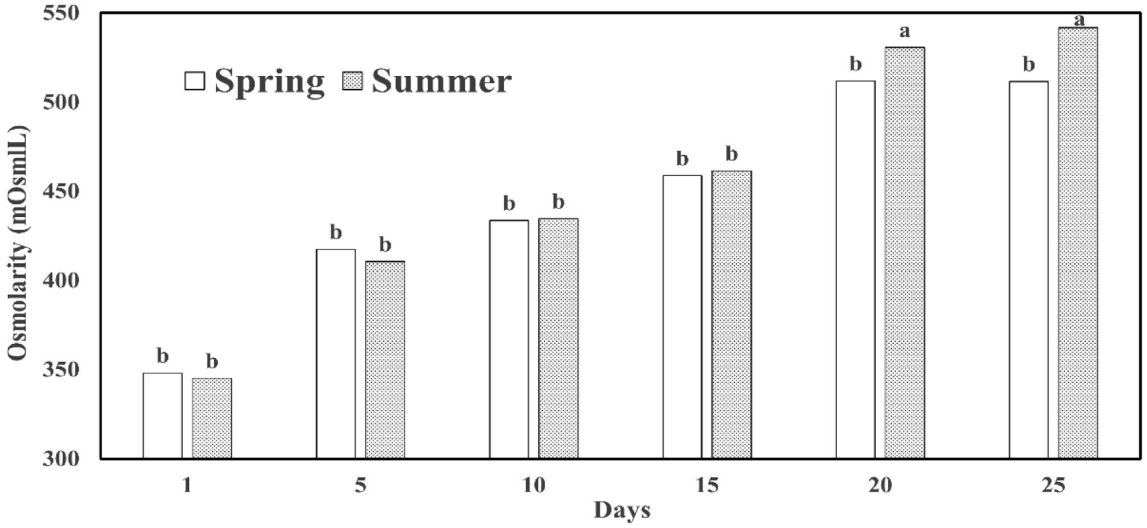

Figure 1. Overall mean of Osmotic concentration of three *Apis mellifera* races (*A. m. carniac*, *A. m. ligustica*, *and A. m. jemenitica*) workers 1–25 days after emergence during spring and summer seasons 2015, Riyadh, Saudi Arabia.

Table 3
Total increase (%) of osmotic concentration (mOsm/L) from day 1 until 25th day after emergence for all three *Apis mellifera* races (*A. m. carniac*, *A. m. ligustica*, *and A. m. jemenitica*) during spring and summer of 2015 in Riyadh, central Saudi Arabia.

Workers age ($n = 30$)	Osmolarity (mOsm/L) (Mean)	
	Spring	Summer
1 day	347.99	344.90
5 days	418.35	406.50
10 days	433.55	434.44
15 days	458.61	461.30
20 days	511.72	527.50
25 days	511.52	541.67
Total increase %	41.5%	48.3%

Table 4
Osmotic concentration of young and old drones in three *Apis mellifera* races (*A. m. carniac*, *A. m. ligustica*, *and A. m. jemenitica*) in spring, 2015, Riyadh, central Saudi Arabia.

Drones age ($n = 5$)	Osmolarity mOsm/L (Mean ± S.D)		
	A. m. ligustica	*A. m. carnica*	*A. m. jemenitica*
Young	394.51 ± 24.09 Ba	398.16 ± 25.84 Ba	389.66 ± 34.18 Ba
Old	434.33 ± 23.99 Aa	425.17 ± 19.12 Aa	412.17 ± 23.70 Aa

*Means followed by the same letter are non-significant at 5% ($p \leqslant 0.05$) level of probability (age = AB, races = ab).

Table 5
Osmotic concentration of young and old drones in three *Apis mellifera* races (*A. m. carniac*, *A. m. ligustica*, *and A. m. jemenitica*) in summer 2015, Riyadh, central Saudi Arabia.

Drones age ($n = 5$)	Osmolarity mOsm/L (Mean ± S.D)		
	A. m. ligustica	*A. m. carnica*	*A. m. jemenitica*
Young	446.25 ± 15.35 Ba	410.50 ± 17.74 Bb	416.75 ± 18.93Bb
Old	499.51 ± 28.82 Aa	481.75 ± 8.37 Ab	488.51 ± 101Ab

*Means followed by the same letter are non-significant at 5% ($p \leqslant 0.05$) level of probability (age = AB, races = ab).

the Italian and Carniolan bee races. Simultaneously, the native bees showed lower rates of body weight loss after exposure to indirect sun light. These observations could be consequences of better water regulation under stressful heat conditions.

After emergence, honey bee workers remain inside colony for about 15 days and perform various duties such as nursing, brood, cleaning and constructing the combs, and feeding the queen as well. They normally start foraging at approximately 15 days of their ages (Seeley, 1995). We found that the switch from nursing to foraging corresponds to an increase in osmolarity, which corresponds to earlier results of Leonhard and Crailsheim (1999) that the decline in osmolarity occurs in drones when they start orientation flights or mating flights. More carbohydrates are required for foragers than nurse bees because of intense flight activity (Crailsheim et al., 1994). Furthermore, foragers have different needs for water balance, which might be due to the need to reduce weight of water because of flight activity. These demands on foragers could underlie the increased hemolymph osmolarity in honey bee foragers. Cohen (1984) and Riddle (1986) reported that exposure to stress and dehydration increases osmolarity in insects.

Our results showed significant differences between overall spring and summer hemolymph osmotic pressure only after 20 and 25 days. This could be explained by the more exposure to high temperature in foragers relative to nurse bees, which remain in the colony at much lower temperature. Hemolymph osmotic pressure increases in other insects such as silk worm, *Bombyx mori* with age and exposure to harsh environment (Nakayama, 1991).

We observed that differences in osmotic concentration between honey bee races were not significantly different at all time periods after emergence except at 25 days in spring, and one and 25 days in summer at which *A. m. ligustica* showed significantly higher values as compared to the other two races. This difference could be either due to differences in switching from nursing to foraging behavior or possibly due to the less adaptation of the Italian bees to the harsh environmental conditions of central Saudi Arabia. *A. m. jemenitica* and *A. m. carnica* workers exhibited similar timing in foraging activities (Alqarni et al., 2014). In another study Alqarni (2010) reported that Italian race *A. m. ligustica* showed significantly lower values for emerging and mating queens and some queens did not lay eggs as compared to *A. m. carnica* and *A. m. jemenitica* queens, which could be related to physiological adaptation to the harsh environmental conditions. Furthermore, exotic honey bee races (*A. m. ligustica and A. m. carnica*) showed higher responses to water than the native race, which might also be due to heat stress (Ali et al., in preparation).

In all three honey bee races osmotic concentration was above 530 mOsm/L after 25 days, which is higher than that recorded in hymenopterous wasps such as the spider wasp *Pepsis formosa* 447 mOsm/L (Punzo, 1990). In insects, osmotic concentration varies within species and even between males and females. Natochin and Parnova (1987) tested osmolarity of 22 insects species from 5 orders. The osmolarity of their hemolymph varied from 319 to 421 mOsm/L, which is lower than what we reported for honey bees. Generally, other insects have lower hemolymph osmolarity ranged from 310 to 532 mOsm/L as mentioned by Buck (1953), lower than reported in honey bee workers and drones.

Leonhard and Crailsheim (1999) reported different osmolarity between honey bee drones and workers. Drones have generally lower osmolarity as they remain inside the colony. However, increased osmolarity was found in drones aged older than 15 days. They reported that drone osmolarity ranged from 334 ± 41 to 532 ± 38 mOsm/L. The lowest value was recorded at day 1 after emergence while the highest value was recorded in drones at 25 days after emergence. Similarly, we recorded that young drones have significantly less osmolarity as compared to old drones. Young drones remain inside the colony while the old drones perform orientation flights, exposing them to environmental stresses and high energy requirements. Other studies of Schneider and Crailsheim (1994) stated that this increase in osmolarity was related to flight activity of drones taking mating flights. They found that corresponding hemolymph volume was significantly lower in drones after flight activity compared to those drones inside colony. The total percentage of increase in drones' osmolarity from the 1st to the 25th day after emergence reported by Leonhard and Crailsheim (1999) (40%) was near to that of spring (41.5%) and summer (48.3%) for all three races in our study during the same time periods.

Osmolarity has a significant effect on nutrient transportation. In stressful conditions, honey bee tissues require nutrients and water, causing an increase osmotic concentration (Crailsheim, 1988). Therefore, it could be suggested that health of honey bee colonies could be assessed through monitoring their osmotic concentration.

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