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Diurnal and Seasonal Flight Activity of the Honeybee, *Apis mellifera* L, and its Relationship with Temperature, Light Intensity and Relative Humidity in the Savanna of Northern Nigeria

By Usman H Dukku, Zecarias Russom & Albert G Domo
Abubakar Tafawa Balewa University, Nigeria

Abstract - This study was carried out at Bauchi (10°19'N; 09°50'E; Elevation 520 m), Nigeria, and it was the first of its kind in the country. Number of foraging bees returning to the hive was recorded for five minutes, at intervals of 30 minutes, and used as an indicator of flight activity. Temperature, light intensity and relative humidity were recorded concurrently. Two peaks of flight activity (one just before sunrise and the other just before sunset), separated by a period of very low activity, were observed in the dry season. However, in the rainy season flight activity was high throughout the day, declining progressively towards sunset. Though there was no significant correlation ($P>0.05$) between flight activity and temperature at 25°C, an inverse and highly significant correlation ($r=-0.75$; $P<0.01$) was found between them at 35°C. Similarly, an inverse and highly significant correlation ($r=-0.87$; $P<0.001$) was observed between flight activity and light intensity.

Keywords : savanna, flight activity, foraging activity, apis mellifera, honeybee, temperature, light intensity, relative humidity, nigeria.

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DIURNAL AND SEASONAL FLIGHT ACTIVITY OF THE HONEYBEE, APIS MELLIFERA L, AND ITS RELATIONSHIP WITH TEMPERATURE, LIGHT INTENSITY AND RELATIVE HUMIDITY IN THE SAVANNA OF NORTHERN NIGERIA

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Diurnal and Seasonal Flight Activity of the Honeybee, *Apis mellifera* L, and its Relationship with Temperature, Light Intensity and Relative Humidity in the Savanna of Northern Nigeria

Usman H Dukku [α], Zecarias Russom [σ] & Albert G Domo [ρ]

Abstract - This study was carried out at Bauchi (10°19'N; 09°50'E; Elevation 520 m), Nigeria, and it was the first of its kind in the country. Number of foraging bees returning to the hive was recorded for five minutes, at intervals of 30 minutes, and used as an indicator of flight activity. Temperature, light intensity and relative humidity were recorded concurrently. Two peaks of flight activity (one just before sunrise and the other just before sunset), separated by a period of very low activity, were observed in the dry season. However, in the rainy season flight activity was high throughout the day, declining progressively towards sunset. Though there was no significant correlation ($P>0.05$) between flight activity and temperature at 25°C, an inverse and highly significant correlation ($r=-0.75$; $P<0.01$) was found between them at 35°C. Similarly, an inverse and highly significant correlation ($r=-0.87$; $P<0.001$) was observed between flight activity and light intensity. No correlation ($r = 0.275$; $P = 0.441$) was found between flight activity and relative humidity. The observed differences in flight activity appeared to be due to environmental factors such as temperature, light intensity and, most importantly, availability of forage rather than the genetic characteristics of the bee.

Keywords : savanna, flight activity, foraging activity, apis mellifera, honeybee, temperature, light intensity, relative humidity, nigeria.

I. Introduction

The honeybee, *A. mellifera*, depends wholly on plants for food. Honeybee workers make thousands of visits to flowers in order to collect nectar and pollen. They also collect propolis from plants, which they use, among other things, in plastering the interior of their nests. Water is collected to dilute honey and to cool the nest during hot weather. Thus, a honeybee colony necessarily lives a busy life. However, the level of activity depends on a number of factors, such as weather, availability of forage and size of the colony. People who work with bees, such as beekeepers and researchers, need to know the pattern of activity of their bees so that they can work at the appropriate time. In the same vein, this knowledge can be used to advise farmers on the appropriate time to spray their crops to avoid killing these beneficial insects while they pollinate these crops.

Investigations into the pattern of activity of various races of *A. mellifera,* in different parts of the world, reveal a lot of variation. For example, Woyke (1992) reported a single peak (in the morning) and two peaks (one after sunrise and one before sunset) of foraging activity for, *A.m. adansonii*, in the rainy and dry seasons, respectively, in Ghana. Similarly Fletcher (1978) reported one peak of activity for *A.m.scutellata* before sunrise in S Africa. On the other hand, Silva and de Jong (1990) reported three peaks (at 7.00, 14.00 and 16.00 h) of activity for Africanized bees in Brazil. Gary (1967) associated the U-shaped pattern of foraging activity he obtained to the high temperature and low humidity conditions of the desert environment. Danka and Beaman (2007), working in the U.S.A., found that Russian and Italian bees had similar flight activity at any given colony size, temperature, or time of day. Flight increased linearly with rising emperatures and larger colony sizes.

The objective of the study was to determine, for the first time, the pattern of diurnal flight activity of different colonies of the indigenous honeybee, *A. mellifera*, and its relationship with seasons, temperature, light intensity and relative humidity, in the Sudan savanna vegetation zone of northern Nigeria.

II. Materials and Methods

a) The Study Area

The study was carried out at Bauchi (9°50 E; 10°19 N; 620 m Above Sea Level) in northern Nigeria in the Sudan (dry) savanna, a belt of vegetation that extends from Senegal to Sudan (Hepburn and Radloff, 1998). The average annual temperature and rainfall are

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25.4°C and 981 mm, respectively. There are two main seasons: a rainy season from May to October and a dry season from November to April (Dukku, 2003).

b) Diurnal Flight Activity

In order to determine the daily flight (or foraging) activity, foraging bees, returning within five minutes, were counted manually, with a tally counter, at the hive entrance, at intervals of 30 minutes, from dawn to dusk. The mean of the two counts within each hour was then used as a measure of flight activity of the colony for that hour. Temperature, light intensity and relative humidity were measured concurrently. In order to obtain seasonal foraging patterns, observations were made on five randomly-picked days for each of the rainy and dry seasons.

c) Data Analysis

Correlation analysis, t-test and one-way analysis of variance (ANOVA) were carried out on the generated data, as appropriate, using the statistical software, MINITAB 15 (Anonymous 2007).

III. Results

a) General Observations

Foraging commenced at dawn, and continued throughout the day, although at varying levels of intensity, irrespective of the honeybee colony or the season, and ended at dusk. Foraging ceased between 15 and 25 minutes after sunset when the intensity of light was 3 or 4 lx. Returning foragers flew straight into the hive or, occasionally, hovered for a while before entering: In either case the landing board was not used. Sometimes they missed the entrance and struck the hive or its support and then crawled in. Thin clouds did not prevent foraging but thick ones did. Bees were observed foraging during light rainfall.

b) Diurnal Flight Activity of Honeybee Colonies within Seasons

Figures 1 and 2 show the pattern of flight activity of two honeybee colonies (#Y1H and Y3H) located at the same site in the dry and rainy seasons, respectively. As could be seen from these figures, the colonies had a similar pattern within each season, though the patterns of the same colony differed between seasons.

c) Diurnal Flight Activity of Honeybee Colonies between Seasons

Flight activity of *A. mellifera* in the dry and rainy seasons is presented in Figure 3. The mean number of returning foragers for the dry and rainy seasons was 258 ± s.e. 83 (n = 13) and 450 ± s.e. 58 (n = 13) per hour, respectively. The difference between these two means was not significant (P>0.05). However, the activity of the bees in the dry season was more variable, with a coefficient of variation (CV) of 115.5%, than in the rainy season which had a CV of 83.6%. By contrast, the rainy season had high flight activity at 08.00 h (mean number or foragers = 715) and 9.00 h (mean number or foragers = 709) with a steady decline thereafter towards the evening.

d) Effect of Temperature on Flight Activity

There was no significant correlation (r = +0.311; P = 0.30) between temperature and number of returning foragers on 24th October, 1999 when temperature averaged 24.7±s.e.0.8°C (n=13) and ranged from 19 to 28°C. On 5th February, 2000 there was a significant, albeit weak, inverse correlation (r = -0.575; P = 0.04) between these two variables at an average temperature of 23.1±s.e.1.3°C (n=13; range= 14 - 29°C). However, on 3rd April, 2011, there was a strong inverse correlation (r = -0.748; P= 0.003) between temperature and number of foragers at an average temperature of 34.7±s.e.1.6°C (n=13; range= 21 - 41°C). A one way analysis of variance (ANOVA) revealed a highly significant (P < 0.001) difference between these three mean temperatures and Tukey's range test further revealed that the means for 24th October (24.7°C) and 5th February (23.1°C) were the same, while that of 3rd April (34.7°C) was different from either of them. Therefore we concluded that at about 25°C, temperature had no effect on foraging activity of *A. mellifera*, but at about 35°C, it had a negative one (Figure 4).

e) Effect of Light Intensity on Flight Activity

There was a very highly significant and inverse relationship (r = -0.87; P < 0.001) between flight activity and intensity of light. The point of determination (r2 x 100) was 75.5%, meaning that about 76% of the variation in flight activity could be explained by light intensity. Moreover, the scatter diagram for this relationship (Figure 5) shows that the relationship was continuous and biologically meaningful.

f) Effect of Relative Humidity on Flight Activity

There was no significant correlation (r = 0.275; P = 0.441) between flight activity and relative humidity.

IV. Discussion

As can be seen in Figures 1 and 2, while the pattern of foraging of *A. mellifera* in the study area does not differ among colonies in the same season, it differs between seasons even for the same colony. In a similar study on *A.m. adansonii* in Ghana, Woyke (1992) found that the pattern of activity, in different zones, did not differ much within each season but the pattern of activity, in different zones, differed much between seasons. This suggests that the pattern is influenced by the environment rather than the genetic makeup of the honeybee colonies. Woyke *et al* (2003) in their study on *A. mellifera, A. laboriosa* and *A. dorsata*, in India, concluded that the environmental conditions in which

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the bees were living for longer period influence their behavior more than their phylogenic relationship. Similarly, Danka and Beaman (2007), working in the U.S.A., found that Russian and Italian bees had a similar flight activity at any given colony size, temperature, or time of day.

The most important environmental factor influencing the pattern of activity of *A. mellifera* appears to be the availability of forage. The dry season pattern, reported in this study, is explained by the dominance of one or a few plants that release nectar and/or pollen at night. Honeybees, being diurnal, exploit these resources near sunrise and sunset times vigorously. In the morning, when they deplete the resources, they fall back on the scanty resources that may be available or they collect water to cool their nests. In the rainy season, on the other hand, forage is usually available throughout the day from a variety of annuals, including crops. For example, the graphs in Figure 1 follow, very closely, the pattern of foraging on *Vitellaria paradoxa*, during whose bloom they were recorded. As reported by Dukku (2010a), honeybees forage on this plant in large numbers from sunset to well into dusk and again from dawn to well after sunrise. Similarly, the pattern in Figure 2 follows, very closely, the pattern of foraging of *A. mellifera* on two dominant plants blooming at the time of the study: *Acacia ataxacantha* and *Guiera senegalensis.* Dukku (2003; 2010b) reported that *A. mellifera* forages on these two plants throughout the day. Furthermore, our finding in this study that flight activity was less variable in the rainy season than in the dry season suggests that the activity of *A. mellifera* is controlled by the availability of forage. Similarly, Pierrot and Schlindwein (2003) concluded that resource availability of the surrounding vegetation seems to be the major factor in defining the forager activities of the stingless bee, *Melipona scutellaris* on a given day.

Our finding that at about 25°C, temperature had no effect on foraging activity of *A. mellifera* agrees with earlier findings. For example, Woyke *et al* (2003) found no correlation between temperature and number of foragers at 18 - 21°C though there was a highly positive correlation at 10 - 16°C. The inverse correlation between temperature and flight activity at about 35°C, reported in this study, agrees with Woyke's (1992) report that at temperatures above 30°C, the number of foragers decreases with the increase in temperature. According to Roman and Weryszko (2006) a temperature of 16-26°C is optimal for foraging by *A. mellifera.*

The inverse correlation between intensity of light and flight activity of *A. mellifera* reported in this study may be indirect or due to the effect of a third factor such as temperature.

V. Conclusion

In conclusion, while recognizing the need for further investigation into this subject, we believe that the availability of forage is the main factor deciding the daily pattern of flight activity of *A. mellifera* in our area of study.

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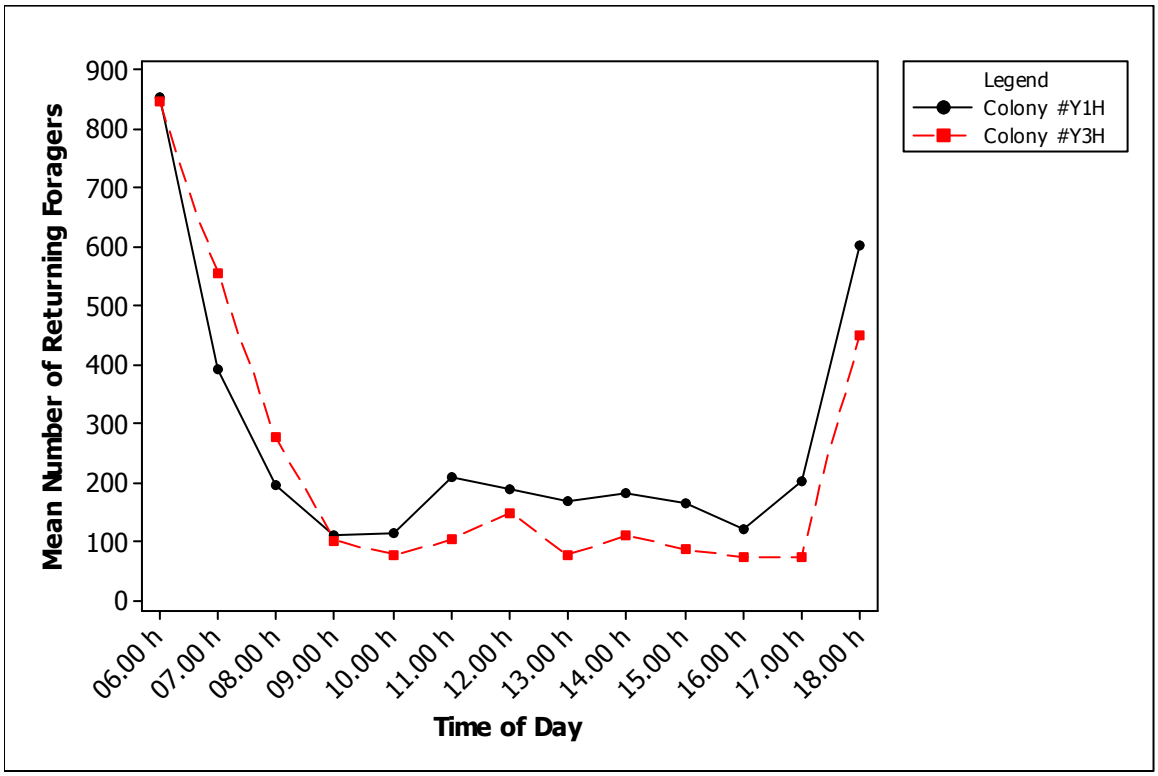

Figure1 : Diurnal flight activity of two honeybee colonies on 5th February 2000 (dry season). The mean number of returning foragers for every hour was obtained by taking the average of two counts taken, during a period of 5 minutes, at the beginning of the hour and 30 minutes later

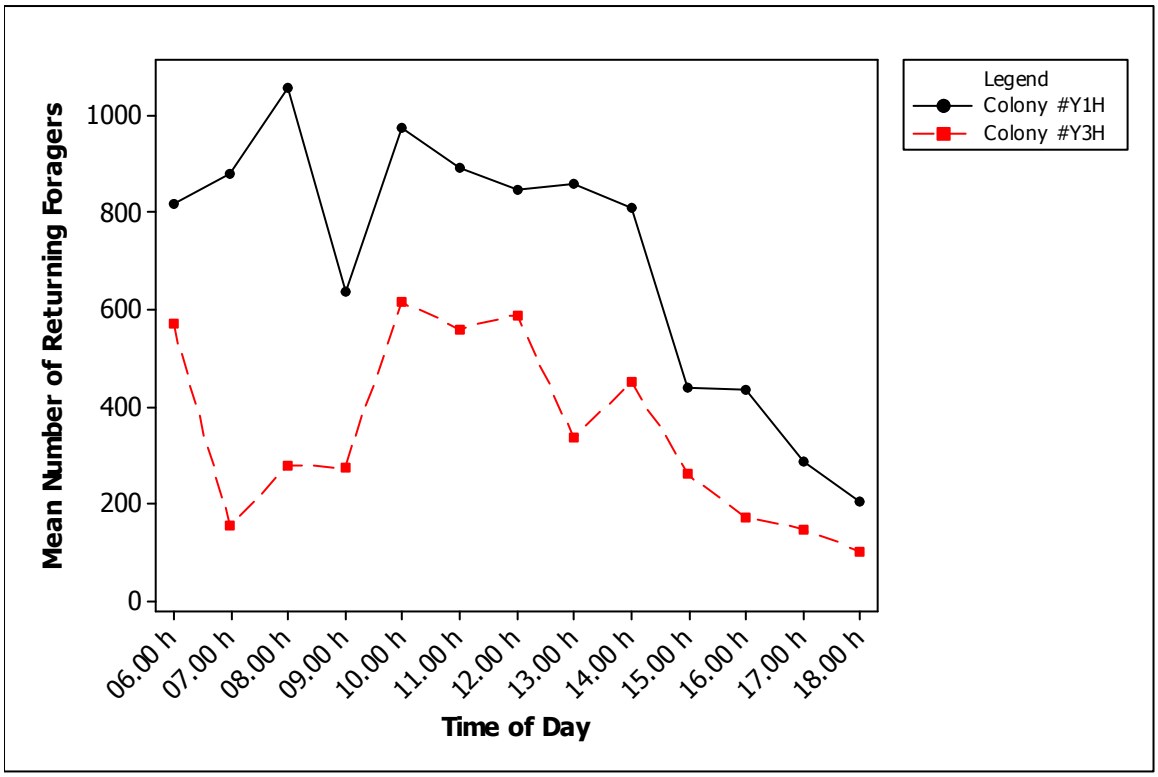

Figure 2 : Diurnal flight activity of two honeybee colonies on 20th August 2000(rainy season). The mean number of returning foragers for every hour was obtained by taking the average of two counts taken, during a period of 5 minutes, at the beginning of the hour and 30 minutes later

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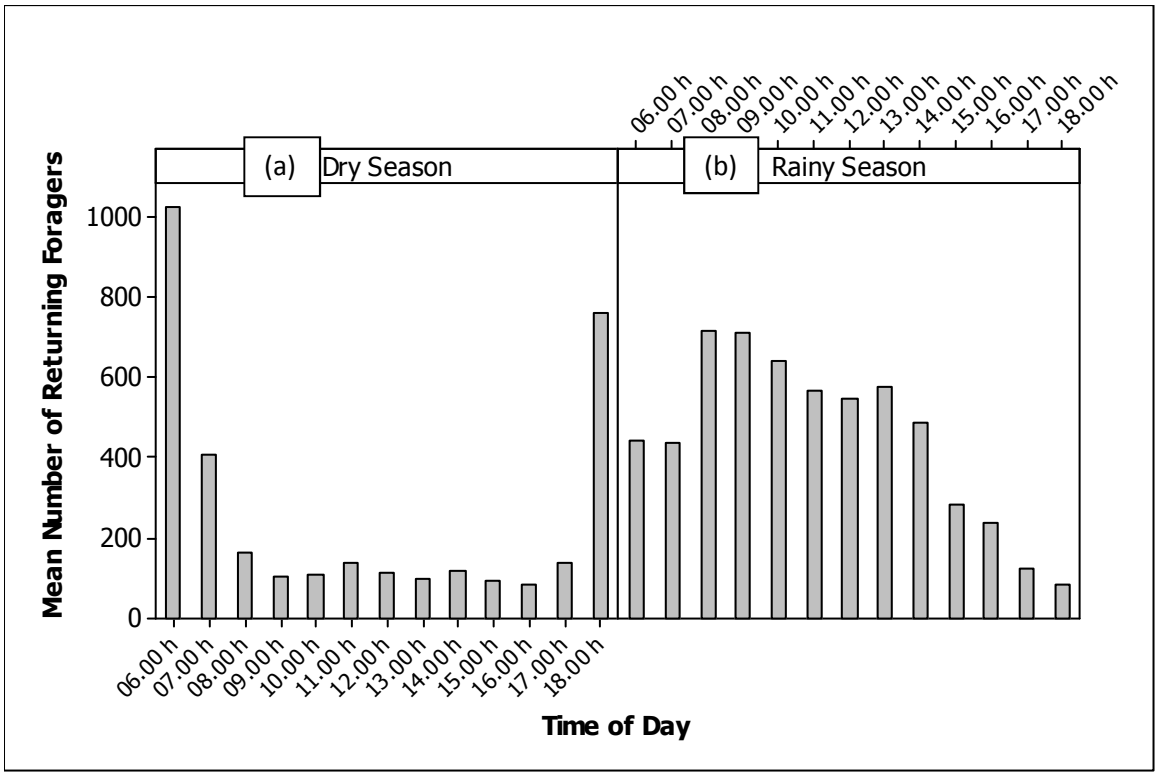

Figure 3 : Diurnal flight activity of honeybee colonies in (a) dry and (b) rainy seasons. The mean number of returning foragers for every hour was obtained by taking the average of two counts taken, during a period of 5 minutes, at the beginning of the hour and 30 minutes later. For each season data of hourly observations taken on five different days were used

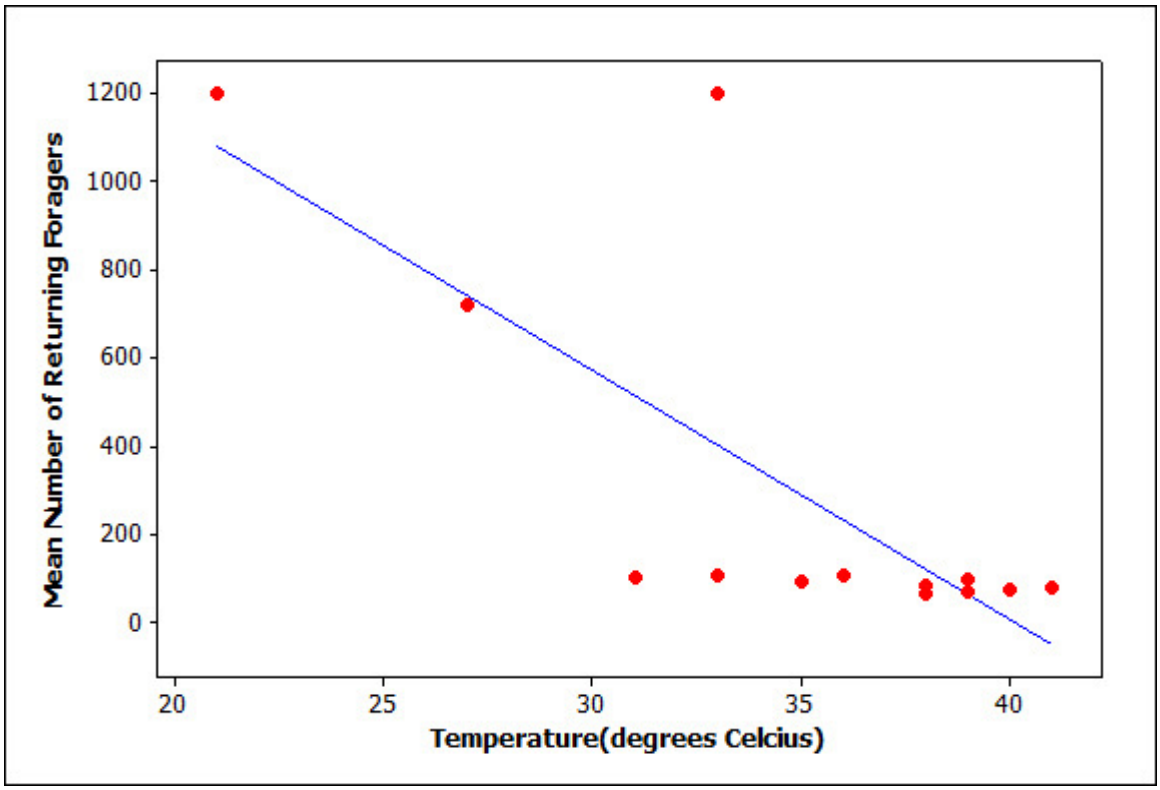

Figure 4 : Relationship between temperature and flight activity of honeybees on 3rd April 2011(mean temperature = 34.7˚C)

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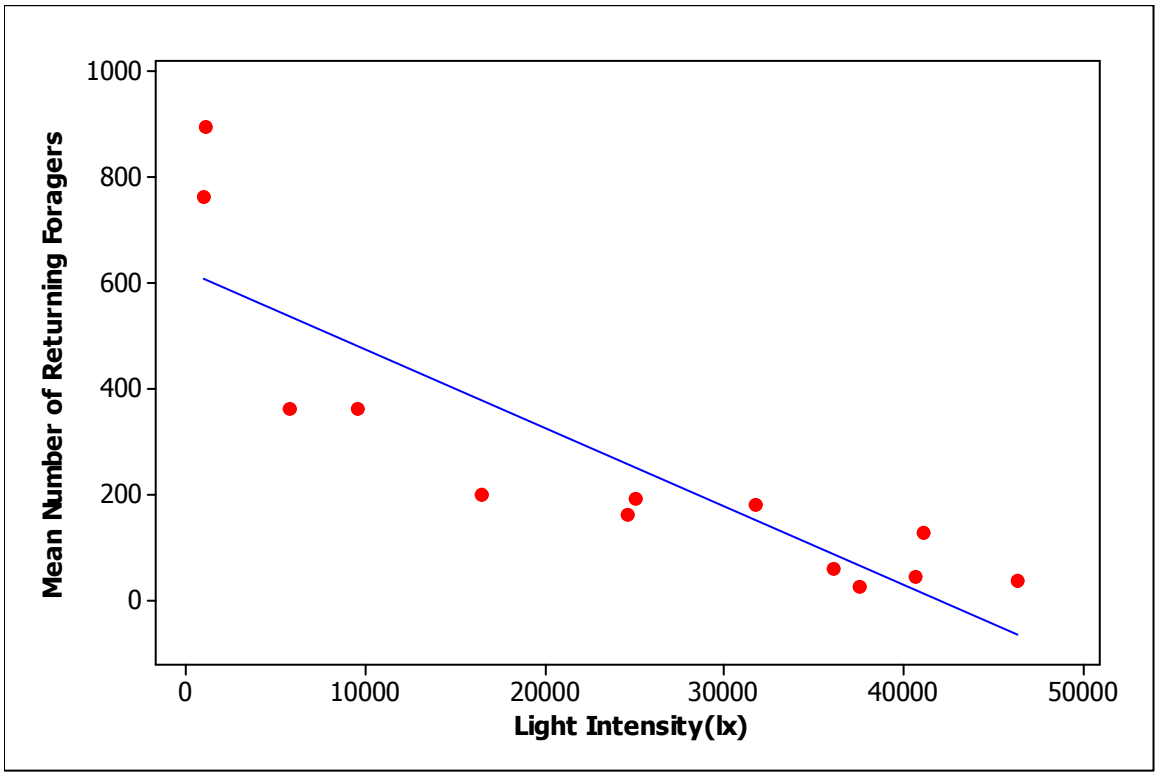

Figure 5 : Relationship between intensity of light and flight activity of honeybees

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Investigations on the Physic-Chemical Parameters and Bacterial Occurrence in Four Water Bodies of Bhopal, India

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Abstract - This study was carried out in four water bodies of Bhopal for a period of 2 years. Monthly changes in physicchemical parameters such as air temperature, water temperature, total dissolved solids, hydrogen-ion concentration, dissolved oxygen, free carbon dioxide, phenolphthalein alkalinity, total alkalinity, chloride, calcium hardness and total hardness along with bacterial occurrence were studied. The results indicated changes in physicchemical parameters and occurrence of bacteria depending on the different seasons of the year.

Keywords : physic-chemical parameters, water bodies, monthly changes.

GJSFR-C Classification : FOR Code: 250499, 091101

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Investigations on the Physic-Chemical Parameters and Bacterial Occurrence in Four Water Bodies of Bhopal, India

Najeeb Parvez [α], MS. A. Mudarris [σ], Ishrat Mohi-ud-din [ρ] & T. A. Qureshi [ω]

Abstract - This study was carried out in four water bodies of Bhopal for a period of 2 years. Monthly changes in physic-chemical parameters such as air temperature, water temperature, total dissolved solids, hydrogen-ion concentration, dissolved oxygen, free carbon dioxide, phenolphthalein alkalinity, total alkalinity, chloride, calcium hardness and total hardness along with bacterial occurrence were studied. The results indicated changes in physic-chemical parameters and occurrence of bacteria depending on the different seasons of the year.

Keywords : physic-chemical parameters, water bodies, monthly changes.

I. Introduction

The physic-chemical parameters are of paramount importance for all the organisms living in water. Many researchers have carried out studies on these parameters in different places in India (Pant and Sharma, 1985; Pataki, 1990; Agawam, 1993; Katarina, *et al.*, 1994; Katarina and Irbil, 1995; Magarey *et al.*, 2006).

Bhopal, the city of lakes, is situated at 23°16' N latitude and 77°26' E longitude. It possesses a number of small and large water bodies including Hate knead reservoir, Lower Lake, Sapura Lake and Larrup reservoir. Hate knead reservoir is situated at 26°16' N latitude and 77°30' E longitude above 460m MSL. Lower lake is situated at 23°16' N latitude and 72°25' E longitude and is one of the twin lakes of Bhopal. Shah pure lake has a surface area of 0.96 sq km. This lake is situated at 23°12' 00" N latitude and 77°25' 30" E longitude. Larrup reservoir is situated at about 10 km away from Bhopal city and lies at 23°11' 45" N latitude and 77°28' 50" E longitude.

Aquatic environment is inhabited with numerous species of bacteria, many of which are responsible for causing dreaded diseases in fishes. Eutrophic water provides favorable habitat for their growth and proliferation because of the availability of nutrients and organic materials (Plumb, 1994). (Koyama and Taming, 1967; Chen, 1968; Fonder, 1969 and Jones, 1970) observed the decline in number and growth of bacteria during summer months which they considered due to high temperature and low concentration of nutrients. (Plumb, 1994) reported that moderate level of temperature is suitable for the growth and development of bacteria.

The present study was, therefore, conducted to investigate the physic-chemical parameters and bacterial occurrence keeping in view the different seasons of the year.

II. Materials and Methods

Important physic-chemical parameters like temperature, total dissolved solids, hydrogen-ion concentration, dissolved oxygen, free carbon dioxide, alkalinity, chloride and hardness of these water bodies were analyzed by the procedures of (APHA, 1995; Goblet roman, 1978 and Wetzel, 1979). Wherever necessary, the DREL / 2000 spectrophotometer, USA was also used for the purpose of water analysis.

For the purpose of isolation of bacteria from water, the samples were collected in sterilized bottles, once every month throughout the study period from five different locations of each water body. The isolation of bacteria from water samples was done following the methods of (Speck, 1976 and Collins and Lynne, 1985). The samples collected were serially diluted and each diluted sample was inoculated in the plates having different media by surface spread method (Speck, 1976). Upton 10 typical colonies selected from each duplicate set of plates were purified, confirmed and differentiated to the genus level.

III. Results and Discussion

During the study period, marked changes were observed in the temperature of all the four water bodies under investigation. Atmospheric temperature ranged between 18.0 to 35.0 °o near Hate knead reservoir, 17.0 to 35.0 °o near Lower lake, 18.0 to 35.0°C near Sapura lake and 18.0 to 35.0°C near Larrup reservoir. The water temperature of Hate knead reservoir, Lower lake, Sapura lake and Larrup reservoir ranged between 20.0 to 30.0°C, 20.0 to 31.0°C, 20.0 to 30.0°C and 20.0 to 30.0°C, respectively. The occurrence of bacterial

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species showed negative relationship with atmospheric and water temperature in all the water bodies except Larrup reservoir where it showed positive relationship. It has been observed that the moderate temperature was more conducive for the occurrence of aquatic bacteria. This finding gets support from the work of (Plumb, 1994; Rodgers and Burke, 1981; Roberts *et al.*, 1986 and Virginal, 1992).

Total dissolved solids ranged between 86.0 to 135.0 mg/l in Hate iced reservoir, 104.0 to 198.0 mg/l in Lower Lake, 86.0 to 135.0 mg/l in Sapura lake and 103.0 to 172.0 mg/l in Larrup reservoir. The occurrence of bacterial species indicated negative relationship with total dissolved solids in Lower lake and Larrup reservoir and positive relationship in Hate iced reservoir and Sapura lake.

pH ranged between 7.5 to 9.0 in Hate knead reservoir, 7.3 to 9.0 in Lower lake, 7.5 to 9.0 in Sapura lake and 7.0 to 8.4 in Larrup reservoir. The occurrence of bacterial species indicated negative relationship with pH in all the water bodies except Hate iced reservoir where it showed positive relationship.

Dissolved oxygen was found to range between 6.4 to 9.1 mg/l in Hate knead reservoir, 5.0 to 9.4 mg/l in Lower lake, 6.4 to 9.1 mg/l in Sapura lake and 5.4 to 10.0 mg/l in Larrup reservoir. Higher dissolved oxygen values observed during winter were due to low temperature and during summer due to the photosynthetic activities. The same have also been the observations of (Sir invasion, 1996; Sashay and Sinhala, 1969; Agawam *et al.*, 1976 and Ray, 1978) in this regard. (Roberts *et al.*, 1986) have reported occasional low oxygen concentrations in several organically enriched environments. The occurrence of bacterial species indicated positive relationship with dissolved oxygen in all the water bodies except Larrup reservoir where it showed negative relationship.

Free carbon dioxide was present in Hate iced only in July, 2004 and 2005. It ranged between 4.6 to 14.9 mg/l in Lower Lake, 9.5 to 19.2 mg/l in Sapura lake and 9.2 to 19.3 mg/l in Larrup reservoir. The occurrence of bacterial species showed positive relationship with free carbon dioxide in Sapura Lake and Larrup reservoir; negative relationship in Lower Lake and no relationship at all in Hate knead reservoir. Phenolphthalein alkalinity ranged between 6.3 to 15.3 mg/l in Hate iced reservoir, 6.5 to 18.2 mg/l in Lower Lake and 6.4 to 10.2 mg/l in Larrup reservoir. The occurrence of bacterial species showed positive relationship with phenolphthalein alkalinity in Lower lake and Larrup reservoir; negative relationship in Hate knead reservoir and no relationship at all in Sapura Lake.

Total alkalinity ranged between 60.0 to 186.0 mg/l in Hate iced reservoir, 110.0 to 262.0 mg/l in Lower Lake, 60.0 to 186.0 mg/l in Sapura Lake and 124.0 to 210.0 mg/l in Larrup reservoir. The occurrence of bacterial species showed negative relationship with total alkalinity in all the four water bodies.

Chloride values ranged between 14.0 to 35.0 mg/l in Hate knead reservoir, 18.5 to 56.0 mg/l in Lower Lake, and 14.0 to 35.0 mg/l in Sapura Lake and 25.0 to 38.0 mg/l in Larrup reservoir. Higher chloride values were observed in Lower Lake as compared to those of Hate iced reservoir, Sapura Lake and Larrup reservoir. Comparatively, lower values were obtained during monsoon and higher during summer months. Similar seasonal trend of fluctuation in chloride values is also reported by (Paha and Mohr okra, 1966; Mathew, 1969 and Agawam *et al.*, 1976). Higher concentration of chloride is an indicator of pollution due to high organic waste of animals (Thresh *et al.*, 1944). The occurrence of bacterial species exhibited negative relationship with chloride in all the four water bodies.

Calcium hardness ranged between 85.0 to 138.0 mg/l in Hate kneads reservoir, 71.0 to 167.0 mg/l in Lower Lake, and 85.0 to 139.0 mg/l in Sapura Lake and 69.0 to 174.0 mg/l in Larrup reservoir. The occurrence of bacterial species showed negative relationship with calcium hardness in all the water bodies except Lower Lake where it showed positive relationship.

Total hardness ranged between 110.0 to 240.0 mg/l in Hate kneads reservoir, 101.0 to 240.0 mg/l in Lower Lake, 154.0 to 243.0 mg/l in Sapura lake and 115.0 to 298.0 mg/l in Larrup reservoir. The occurrence of bacterial species showed negative relationship with calcium hardness in Hate iced reservoir and Sapura lake while it showed positive relationship in Lower lake and Larrup reservoir.

It was observed that the occurrence and abundance of aquatic bacteria varied from time to time depending on the availability of nutrients and prevailing physico-chemical parameters. The bacteria isolated represented mainly the families *Bacillaceae, Cytophagaceae, Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae* and *Streptococcaceae*. Of the total bacteria isolated, *Aeromonas, Acinetobacter*, Bacillus, *Cytophaga, Escherichia, Flexibacter*, Micrococcus, Pseudomonas, Staphylococcus and Streptococcus were the common genera found in all the water bodies.

Abundant occurrence of bacteria was recorded during and after rainy season in all the four water bodies. It might be due to the influx of large volume of sewage loaded with bacteria and other organic material. This finding gets support from the observations of (Fred et al., 1924; Taylor, 1949 and Collins, 1963). Decrease in the number of bacteria is noticed during summer months which might be due to high temperature and low nutrient level. Similar findings are reported by (Koyama and Domino, 1967; Chen, 1968; Fonder, 1969 and Jones, 1970).

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Table 1 : Physic-chemical parameters of Hate iced reservoir (August 2003-July 2005)

Month	AT °C	WT °C	TDS (mg/l)	pH	DO (mg/l)	Free CO_2 (mg/l)	PA (mg/l)	TA (mg/l)	Chloride (mg/l)	CH (mg/l)	TH (mg/l)
Aug	27	28	101	7.5	7.1	0	4.2	110	26	1.5	188
Sep	29	27	113	7.6	6.9	0	6.3	82	24	111	199
Oct	27	26	127	7.9	7.4	0	8.7	62	22	119	210
Nov	24	23	130	8.0	7.7	0	9.5	83	20	133	299
Dec	22	24	119	8.4	7.9	0	11.8	105	16	138	240
Jan	19	21	98	8.6	8.5	0	8.4	111	17	108	191
Feb	21	22	88	8.8	9.1	0	12.5	123	19	103	172
Mar	25	23	94	8.3	6.7	0	9.7	131	23	117	201
Apr	30	27	101	8.1	6.9	0	12.8	145	33	123	213
May	35	29	115	7.9	7.2	0	14.7	179	31	109	194
Jun	31	28	121	7.6	7.5	0	11.3	147	29	96	142
Jul	29	27	130	7.9	7.1	6.4	0	122	28	87	119
Aug	26	25	96	7.6	7.2	0	4.9	116	27	102	182
Sep	28	26	120	7.8	7.0	0	6.8	84	22	108	193
Oct	28	27	131	8.2	7.6	0	9.3	60	19	122	218
Nov	25	24	128	8.4	7.9	0	102	85	17	129	221
Dec	21	23	114	8.7	8.2	0	12.5	109	14	132	237
Jan	18	20	102	8.8	8.6	0	8.3	116	18	105	117
Feb	20	21	86	9.0	9.0	0	12.7	130	21	101	157
Mar	25	23	96	8.6	6.4	0	9.0	139	29	112	204
Apr	29	25	105	8.4	6.8	0	13.1	148	35	128	221
May	34	28	118	8.2	7.0	0	15.3	186	33	106	181
Jun	31	27	123	7.8	7.8	0	12.9	156	30	98	149
Jul	29	26	135	8.2	7.4	4.0	0	130	25	85	110

AT=Atmospheric temperature, WT=Water temperature, TDS=Total Dissolved Solids, DO=Dissolved Oxygen, PA=Phenolphthalein Alkalinity, TA=Total Alkalinity, CH=Calcium Hardness, TH=Total Hardness

Table 2 : Physic-chemical parameters of Lower lake (August 2003-July 2005)

Month	AT °o	WT °o	TDS (mg/l)	pH	DO (mg/l)	Free CO_2 (mg/l)	PA (mg/l)	TA (mg/l)	Chloride (mg/l)	CH (mg/l)	TH (mg/l)
Aug	27	25	115	7.3	5.8	5.2	0	168	52.3	158	208
Sep	28	26	119	7.4	5.4	6.0	0	148	42.4	167	280
Oct	30	27	132	7.7	5.0	0	12.0	115	33.8	122	194
Nov	26	24	124	8.1	6.4	0	7.8	128	28.2	111	167
Dec	22	24	113	8.2	8.2	8.4	0	137	21.5	102	143
Jan	18	21	109	7.4	8.8	10.1	0	143	19.2	88	127
Feb	22	23	104	7.7	9.4	12.8	0	162	23.2	75	109
Mar	28	26	148	8.1	7.2	14.9	0	194	28.8	93	138
Apr	31	28	162	8.5	6.7	0	6.5	235	36.7	106	157
May	35	30	194	8.8	6.1	0	10.2	262	55.6	117	173
Jun	31	27	171	8.3	7.3	0	17.8	213	45.0	132	198
Jul	29	26	138	8.0	6.5	8.6	0	192	51.8	145	201
Aug	26	23	118	7.3	5.9	4.6	0	175	54.0	160	271
Sep	28	26	125	7.6	5.5	6.2	0	154	44.2	155	204
Oct	29	25	136	7.9	5.2	0	12.2	110	38.0	134	199
Nov	25	22	122	8.2	6.4	0	8.0	121	31.5	115	181
Dec	21	23	116	8.0	8.0	8.5	0	128	24.6	100	134
Jan	17	20	112	7.4	8.4	10.4	0	140	18.5	83	121
Feb	21	24	106	7.6	8.8	12.5	0	166	20.0	71	101
Mar	27	25	156	8.0	6.4	14.6	0	197	27.2	89	130

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Apr	30	28	164	8.8	6.3	0	7.0	232	38.0	105	159
May	34	29	198	9.0	6.2	0	10.4	246	56.0	120	182
Jun	32	28	178	8.5	6.8	0	18.2	210	48.2	137	203
Jul	30	27	142	8.2	6.0	8.5	0	196	52.2	149	211

AT=Atmospheric temperature, WT=Water temperature, TDS=Total Dissolved Solids, DO=Dissolved Oxygen, PA=Phenolphthalein Alkalinity, TA=Total Alkalinity, CH=Calcium Hardness, TH=Total Hardness

Table 3 : Physic-chemical parameters of Sapura lake (August 2003-July 2005)

Month	AT °C	WT °C	TDS (mg/l)	pH	DO (mg/l)	Free CO_2 (mg/l)	PA (mg/l)	TA (mg/l)	Chloride (mg/l)	CH (mg/l)	TH (mg/l)
Aug	27	26	92	7.6	7.2	14.9	0	116	27	102	182
Sep	29	27	120	7.8	7.0	16.8	0	84	22	108	193
Oct	27	25	131	8.2	7.6	19.2	0	60	19	122	208
Nov	26	24	128	8.4	7.9	10.3	0	85	17	139	221
Dec	22	24	114	8.7	8.2	11.7	0	109	14	132	215
Jan	19	22	102	8.8	8.6	12.1	0	116	18	105	189
Feb	22	24	86	9.0	9.0	12.6	0	130	21	101	176
Mar	25	23	96	8.6	6.4	13.0	0	139	25	112	201
Apr	29	27	105	8.4	6.8	13.9	0	148	35	128	216
May	35	30	118	8.2	7.0	15.2	0	186	33	106	193
Jun	31	28	123	7.8	7.8	12.5	0	156	30	98	178
Jul	29	27	135	8.2	7.4	11.4	0	130	29	85	154
Aug	28	26	101	7.5	7.1	14.2	0	110	26	105	188
Sep	30	27	113	7.6	6.4	16.3	0	82	24	111	199
Oct	28	24	127	7.9	7.1	18.7	0	62	22	119	210
Nov	25	23	130	8.0	7.7	9.5	0	83	20	133	220
Dec	21	23	119	8.4	7.9	11.3	0	105	16	138	243
Jan	18	20	98	8.6	8.5	11.8	0	111	17	108	195
Feb	20	22	89	8.8	9.1	12.2	0	123	19	103	187
Mar	25	23	94	8.3	6.7	12.7	0	131	23	117	205
Apr	28	26	101	8.1	6.9	13.4	0	145	33	123	212
May	34	29	115	7.9	7.2	14.7	0	179	31	109	199
Jun	32	28	121	7.6	7.5	11.6	0	147	29	96	164
Jul	30	27	132	7.9	7.1	10.2	0	122	28	87	157

AT=Atmospheric temperature, WT=Water temperature, TDS=Total Dissolved Solids, DO=Dissolved Oxygen, PA=Phenolphthalein Alkalinity, TA=Total Alkalinity, CH=Calcium Hardness, TH=Total Hardness

Table 4 : Physic-chemical parameters of Larrup reservoir (August 2003-July 2005)

Month	AT °C	WT °C	TDS (mg/l)	pH	DO (mg/l)	Free CO_2 (mg/l)	PA (mg/l)	TA (mg/l)	Chloride (mg/l)	CH (mg/l)	TH (mg/l)
Aug	29	26	131	7.1	7.4	14.2	0	139	33	147	276
Sep	30	28	157	7.2	6.9	13.5	0	124	29	172	294
Oct	28	26	159	7.5	6.3	16.7	0	146	25	109	183
Nov	25	22	132	7.6	7.9	18.2	0	141	28	99	165
Dec	22	23	119	7.8	7.2	19.3	0	133	30	93	133
Jan	18	20	103	7.5	7.1	15.8	0	147	37	69	115
Feb	21	22	111	7.3	6.9	13.0	0	159	29	102	178
Mar	26	24	116	8.1	6.5	11.5	0	173	33	114	185
Apr	30	27	143	8.4	9.2	0	7.2	182	35	120	215
May	34	29	157	8.3	5.8	0	8.9	163	37	141	254
Jun	32	29	128	7.9	5.4	0	9.7	201	34	156	273
Jul	30	26	117	7.5	6.2	0	7.7	158	35	170	289

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Aug	28	25	124	7.0	7.2	12.7	0	150	28	152	282
Sep	30	26	172	7.3	6.6	10.8	0	130	26	174	298
Oct	27	25	166	7.7	6.4	18.5	0	148	27	118	202
Nov	24	23	148	7.9	8.2	14.9	0	142	28	110	188
Dec	20	22	140	7.5	8.0	16.3	0	130	29	102	175
Jan	19	21	110	7.2	7.6	12.8	0	151	38	75	122
Feb	22	23	122	7.4	7.2	10.0	0	165	27	106	186
Mar	27	25	130	8.3	7.0	9.2	0	180	34	120	219
Apr	31	27	160	8.1	10.0	0	6.4	194	33	124	227
May	35	30	170	8.6	6.0	0	9.8	178	37	140	251
Jun	33	29	140	8.2	5.6	0	10.2	210	30	159	282
Jul	31	28	115	7.2	6.8	0	8.5	170	32	163	291

AT=Atmospheric temperature, WT=Water temperature, TDS=Total Dissolved Solids, DO=Dissolved Oxygen, PA=Phenolphthalein Alkalinity, TA=Total Alkalinity, CH=Calcium Hardness, TH=Total Hardness

Table 5 : Mean and Standard Deviation of various ecological parameters and bacterial species

Parameters	Hate iced reservoir	Lower lake	Sapura lake	Larrup reservoir
Atmospheric temperature	26.38 ± 4.4517	26.96 ± 4.64	26.67 ± 4.459	27.17 ± 4.749
Water temperature	25.0 ± 2.5	25.29± 2.474	25.21 ± 2.449	25.29 ± 2.776
Conductivity	206.5 ± 29.67	278.71± 52.106	210.5 ± 33.29	283.03 ± 27.788
Total dissolved solids	112.17 ± 14.45	137.58 ± 27.28	112.08 ± 14.76	136.25 ± 20.33
Hydrogen-ion concentration	8.18 ± 0.4231	8.0 ± 0.4822	8.18 ± 0.4213	7.69 ± 0.4499
Dissolved oxygen	7.54 ± 0.7076	6.78 ± 1.2024	7.54 ± 0.7076	7.05 ± 1.04008
Free carbon dioxide	-	9.38 ± 3.225	13.34 ± 2.475	14.21 ± 2.97
Phenolphthalein alkalinity	10.22 ± 2.95	11.01 ± 3.962	-	8.55 ± 1.269
Total alkalinity	119.13 ± 32.14	173.83 ± 42.81	119.13 ± 32.14	158.92 ± 22.79
Chloride	24.08 ± 5.88	37.12 ± 12.52	24.08 ± 5.88	31.42 ± 3.817
Calcium hardness	111.54 ± 13.89	118.08 ± 27.58	111.96 ± 14.55	126. 46 ± 29.78
Total hardness	189.5 ± 33.81	174.63 ± 44.84	196.04 ± 20.74	220.13 ± 56.75
Bacteria	6.96 ± 1.6951	7.17 ± 1.95	7.46 ± 4.45	7.04 ± 1.989

Table 6 : Coefficient of Correlation between the occurrence of bacterial species and various ecological parameters

Parameters	Hate iced reservoir	Lower lake	Sapura lake	Larrup reservoir
Air temperature	- 0.23 (1.135)	-0.2247(1.081)	- 0.294 (1.445)	0.1227 (0.5801)
Water temperature	- 0.137 (0.651)	- 0.2603 (1.26)	-0.2191 (1.053)	0.12607 (0.5961)
Conductivity (μmhos/cm)	0.1313 (0.621)	- 0.3135 (1.548)	- 0.0849 (0.399)	-0.0518 (0.2436)
TDS (mg/l)	0.326 (1.622)	-0.4769 (2.545)*	0.0872 (0.410)	- 0.0661 (0.3111)
pH	0.0396 (0.186)	-0.5271(2.90)*	-0.1342 (0.6355)	- 0.3348 (1.666)
Dissolved oxygen (mg/l)	0.338 (1.68)	0.0902 (0.425)	0.389 (1.98)	- 0.5263 (2.90)*
Free CO_2 (mg/l)	-	- 0.285 (1.030)	0.129 (0.6101)	0.2980 (1.16)
Phenolphthalein alkalinity (mg/l)	- 0.3009 (1.411)	0.484 (1.56)	-	0.684 (2.30)
Total alkalinity (mg/l)	- 0.425 (2.20)*	- 0.4795 (2.56)*	- 0.576 (3.31)*	- 0.1396 (0.6614)
Chloride (mg/l)	- 0.2922 (1.433)	- 0.064 (0.3036)	- 0.392 (1.99)	- 0.3095 (1.52)
Calcium hardness (mg/l)	- 0.3032 (1.49)	0.2165 (1.04)	- 0.2042 (0.978)	0.2725 (1.328)
Total Hardness (mg/l)	- 0.3442 (1.719)	0. 1797 (0.857)	- 0.1874 (0.894)	0.2435 (1.177)

*Values are significant at 5 % level

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Increased Serum Homocysteine Level Association with Methylenetetrahydrofolate Reductase C677T and A1298C Mutations in Patients with Cerebral Infarction in the Latvian Population

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Abstract - Background and Objective : A number of studies have previously described elevated levels of homocysteine as an independent coronary heart disease and stroke risk factor. The results of above studies show different data for the methylenetetrahydrofolate reductase (MTHFR) genetic polymorphism and hyperhomocysteinemia, which is the cause of cerebrovascular accident. Purpose of the study was to determine whether there is a link between hyperhomocysteinemia and A1298CC, C677 genotype associated with acute cerebral infarction.

Methods : The prospective study included patients (n=102) with acute cerebral infarction, regardless of its genesis or transient ischemic attack, and patients with a history of cerebral infarction of any age, that correlates with the imaging techniques.

Keywords : cerebrovascular disease; homocysteine; mutation; risk factor; stroke.

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Increased Serum Homocysteine Level Association with Methylenetetrahydrofolate Reductase C677T and A1298C Mutations in Patients with Cerebral Infarction in the Latvian Population

Pauls Auce [α], Anželika Gudreniece [σ], Viktorija Ķēniņa [ρ], Daina Pastare [ω], Gundega Berģe [¥], Linda Piekuse [§] & Andrejs Millers [χ]

Summary - Background and Objective : A number of studies have previously described elevated levels of homocysteine as an independent coronary heart disease and stroke risk factor. The results of above studies show different data for the methylenetetrahydrofolate reductase (MTHFR) genetic polymorphism and hyperhomocysteinemia, which is the cause of cerebrovascular accident. Purpose of the study was to determine whether there is a link between hyperhomocysteinemia and A1298CC, C677 genotype associated with acute cerebral infarction.

Methods : The prospective study included patients (n=102) with acute cerebral infarction, regardless of its genesis or transient ischemic attack, and patients with a history of cerebral infarction of any age, that correlates with the imaging techniques. The control group (n = 34) consisted of patients without a history of cerebrovascular disease, showing no indication of previous strokes according to imaging techniques. Homocysteine was determined using IMMULITE 2000 testing system.

Results : Comparing the both groups, increased homocysteine level association with the cerebrovascular event was not observed (p=0.4). By studying the genetic polymorphism of MTHFR, a statistically significant relationship of elevated homocysteine with C677TT (p=0.15), C677CT (p=0.61) and C677CC (p=0.90) was not detected. Similar results were obtained for A1298 genetic polymorphism.

Conclusion : This study showed that there is no link between hyperhomocysteinemia and MTHFR genetic polymorphism in the investigated population associated with risk of acute cerebral infarction. Taking into consideration the relationship of homocysteine with folic acid and vitamin B12 levels, the next phase of study will include the determination of these two parameters in addition.

Keywords : cerebrovascular disease; homocysteine; mutation; risk factor; stroke.

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I. Introduction

In Latvia mortality rate from ischemic and hemorrhagic stroke is 230/100 000 annually for people aged from 35 to 74 years (1); cerebrovascular diseases is the leading cause of severe disability (2). Healthcare costs due to cerebrovascular diseases in the year 2003 accounted for 21 billion euros in all 25 European Union countries alone (3).

Due to the trend of aging of the European Union's population the topicality of the problem is growing. Solution could be targeted identification of the ischemic stroke risk factors and their following modification. Multiple etiological moments of the cerebral infarction make the solution of these problems difficult. Atherothrombosis and atherosclerosis are important causes of stroke, the most common in the younger age group (4). Atherosclerosis is also indirectly involved in other stroke etiology and pathogenesis stages, for example, like coronary heart disease. A number of studies have previously described elevated level of homocysteine as an independent coronary heart disease (5) and stroke (6) risk factor, and there are indications of its possible special connection with cerebral infarction due to the large blood vessel atherothrombosis (7). However, the situation is not so unequivocal as there are also studies that do not detect such a relationship to cardiovascular diseases (8), (9). Moreover, taking into account the heterogeneity of etiology and pathogenesis of stroke, it is not completely clear how this relates to its sub-types. The situation is further complicated by the fact that right now most studies have not demonstrated the efficacy of folic acid in cerebrovascular and cardiovascular event reduction (10), (11), (12), there is only one meta - analysis, which indicates the contrary (13). Similar is the situation in the B group vitamins combination use in the cardio-and cerebrovascular disease risk reduction - several studies showed no reduction (14), (15), while others showed a positive effect (16), (17), but, for example, one study

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showed that folic acid in combination with B vitamins possibly increases the risk of revascularization following percutaneous coronary intervention (18).

II. Materials and Methods

The research was done in VSIA „Pauls Stradins Clinical Hospital" during the period from 01/01/2011 to 15/12/2011. The study was prospective in nature. The case study group included patients with acute cerebral infarction regardless of its genesis, or transient ischemic attack, as well as patients with a history of cerebral infarction of any age that correlated with the imaging techniques data. The control group consisted of patients without a history of cerebrovascular disease, or episode, excluding patients showing indications of prior cerebrovascular diseases according to the imaging techniques.

The work was received the Ethics Committee of Riga Stradiņš university approval.

Demographic, clinical and paraclinical data were taken from routine questionnaires, which are used in the stroke registry. For the control group was used in the same questionnairy as for the case group, only it was was completed in the modified scale.

Homocysteine was determined using IMMULITE 2000 testing system, which uses solid phase hemiluminiscence enzyme immunoassay test for L-homocysteine quantitativedetermination in plasma and serum. It is based on two cycles – release of the bound homocysteine, and its subsequent conversion to S-adenosyl-L-homocysteine (SAH) and the immune response. Antibodies used in the test system are specific for homocysteine. Elevated homocysteine value was considered to be more than 14.25 mmol / l according to the American Stroke Association Guidelines (19). DNA was isolated from venous blood using standard phenol - chloroform method. Genotyping of C677T and A1298C polymorphisms was done by PCR and RFLP analysis (restrictases HinfI, MboII). The study database was created in EpiData software. For statistically processing the STATA StataCorp (2007) Statistical Software was used: Release 11. College Station, TX: StataCorp. Both descriptive and analytical statistical methods were applied.

III. Results

a) *Demographic Data*

Among 137 of the study subjects 43, 1% (59/137) were men and 56, 9% (78/137) women. Distribution by sex between cases and controls was not statistically significant ($p = 0.512$). In the control group, the age ranged between 45 and 87 years with median of 67 years and quartile boundaries between 59 and 72 years. In the cases group age ranged between 39 and 88 years with a median of 71 years and the quartile limits between 65 and 78 years. The both groups were statistically significantly different by age, $p < 0.01$; the cases group was statistically significantly older.

b) *MTHFR Genetic Polymorphism*

C677 genetic polymorphism results for the cases and control groups are summarized in the 1th table.

A1298 genetic polymorphism represents the 2nd table, where A1298CC - homozygote, which is due to the labile form of MTHFR, A1298AA – a „normal" homozygote.

Homocysteine levels have been measured in 136 individuals, the average rate was 13.2 μmol / l, with a standard deviation of 4.7. In the cases group (102 persons), it ranged from 4.7 to 32.3 μmol / l, and the average level was 13.2 μmol / l with a standard deviation of 4.85. In the control group (34 persons) homocysteine level ranged from 8.2 to 25.2 μmol / l, and the average rate was 13.4 μmol / l with a standard deviation of 4.2. Between the two groups a statistically significant difference was not observed, $p = 0.4$. Relationship of an elevated homocysteine level with cerebrovascular event was not established, OR = 1.04, 95% confidence interval 0.42 to 2.66; $p = 0.9$.

c) *MTHFR and Hyperhomocysteinaemia*

Analyzing the association of an elevated homocysteine level with MTHFR genetic polymorphism no statistically significant relationship with C677TT mutations was observed, $p = 0.15$, such relationship was not found also with the C677CT genotypes, $p = 0.61$ and also with the C677CC – normal homozygote, $p = 0.90$. The 3rd table illustrates the tested relation of C677 genetic polymorphism to elevated serum homocysteine level.

Similar analysis was also done for A1298 genetic polymorphism, where there were found the same results, no statistically significant relationship between hyperhomocysteinemia and A1298CC genotype, $p = 0.09$, heterozygous variant, $p = 0.21$ and the normal homozygote, $p = 0.94$ was detected, that represent 4th table.

IV. Discussion

The paper's findings have already been extensively studied and described before. From our neighbors in the north of Poland: their study has already found no link between the two investigated MTHFR polymorphism variants, in addition the genotype C677TT in the target group was found in 8% cases and A1298CC in 14% cases, which is close to our results, only in this study there was found the link of a variant of C677TT polymorphism with increased level of homocysteine in the serum (20). The study of our other neighbours in Sweden also found no relationship between the C677TT mutation, hyperhomocysteinemia,

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acute cerebrovascular event, internal carotid artery stenosis and folic acid level (22). In this study the TT genotype was present in 10% of both control and cases groups. Another study from a country from our region - Denmark also did not show a direct relation of C677TT genotype with stroke, but did show relation of C677TT genotype with elevated homocysteine level; C677TT genotype was found in the 10.6% and 8.3% persons of the cases and control groups correspondingly (23). Another study, in which target group consisted of younger people with an acute stroke from Poland, also found no relationship between C677TT genotype in this age group, the TT genotype occurred in 12% persons in the stroke group and 10.9% in the control group (24). In Russia the genotype C677TT according to literature occurs in 8.4% of healthy population (25). Without taking into account the small size of the control group, but only considering the genotype frequencies of the target group, it can be concluded that there are no significant differences comparing with our neighbouring countries.

After the last great meta-analysis, at which was assessed the relationship between MTHFR genetic polymorphism, hyperhomocysteinaemia and dietary folic acid quantity, it was concluded that in areas with increased use of folic acid or permanent food enrichment there is no benefit from reducing homocysteine with a purpose to prevent stroke. MTHFR 677C→T polymorphism association with stroke should be investigated in areas with a low folate level (21). In this phase of the study, neither vitamin B_{12}, nor folic acid were measured, this is planned to do in the next phase. The author failed to find large and high-quality population data on the vitamin B_{12} and folic acid levels in the blood of Latvia's population as well; hopefully, we will be able to successfully reply to this question in our next study. In view of the above facts it is not possible to speak certainly on the lack of association between the two investigated genotypes and homocysteine level.

V. Conclusion

The incidence of MTHFR C677 T and C alleles was established, which will be used to calculate the size of the control and cases groups of the next phase of the study. The identical work was done with A1298 A and C alleles. It was found that there is no relationship between the hyperhomocysteinaemia and the studied MTHFR genetic polymorphism in the incorporated population.

VI. Conflicts of Interest

The autors declare no conflicts of interests.

Table 1 : C677 genetic polymorphism in the control and cases groups. C677TT - homozigote that is associated with the MTHFR thermolabile form, C677CC - "normal" heterozigote

	Genotype			
Group	**C677TT**; N=13, 10.2 %	**C677CT**; N=47, 36.7 %	**C677CC**; N=68, 53.1 %	**Total**
Cases	10 (10.1)	33 (33.3)	56 (56.6)	99 (100.0)
Controls	3 (10.3)	14 (48.3)	12 (41.4)	29 (100.0)

Table 2 : A1298 genetic polymorphism in the cases and control groups

	Genotype			
Group	**A1298CC**; N=23, 17,4%	**A1298AC**; N=50, 37,8 %	**A1298AA**; N=59, 44,7 %	Total
Cases	17 (17.0)	40 (40.0)	43 (43.0)	100 (100,0)
Controls	6 (18.8)	10 (31.3)	16 (50.0)	32 (100.0)

Table 3 : C667 genetic polymorphism in relation to the serum elevated homocysteine level

Homocysteine level				
Genotype	**Elevated, N (%)**	**Normal, N (%)**	OR; 95% confidence interval	p value
C677TT (N=13, 10.1%)	11 (12.8)	2 (4.8)	0.33; [0.03 – 1.65]	0.15
C677CT (N=47, 36.7%)	17 (40.5)	30 (34.9)	1.22; [0.53 – 2.77]	0.61
C677CC (N=68, 53.1%)	23 (54.8)	45 (52.3)	1.05; [0.47 – 2.33]	0.90
Total (N=128, 100%)	42 (100%)	86 (100%)		

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Table 4 : Relation of the A1298 genetic polymorphism to elevated serum homocysteine level.

	Homocysteine level			
Genotype	Elevated, N (%)	Normal, N (%)	OR; 95% confidence interval	p value
A1298CC (N=23, 17.4%)	11 (25.6)	12 (13.5)	2,21; [0.79 – 6.07]	0.09
A1298AC (N=50, 37.9%)	13 (30.2)	37 (41.6)	0.61; [0,26 – 1,40]	0.21
A1298AA; (N=59; 44.7%)	19 (44.2)	40 (44.9)	0.97; [0,44 – 2,15]	0.94
Total (N=132; 100%)	43 (100%)	89 (100%)		

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Using of Semi-Empirical Models and Fick's Second Low for Mathematical Modeling of Mass Transfer in Thin Layer Drying of Carrot Slice

By Isa Hazbavi, Seyed Hashem Samadi & Hamid Khafajeh
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Abstract - Drying behavior of carrot slices were studied at 200, 300, 400 and 500 W for constant sample thickness (5mm) in a microwave dryer. By increasing the microwave output powers (200-500 W), the drying time decreased from 17.5 to 9.5 min. The drying process took place in the falling rate period. Six mathematical models for describing the thinlayer drying behavior of carrot slices were investigated. The models were compared based on their coefficient of determination (R2), root mean square error (RMSE) and reduced chi-square ($\chi 2$) values between experimental and predicted moisture ratios. The results show that the Midilli model is the most appropriate model for drying behaviour of thin layer carrot slices. Moisture transfer from carrot slices was described by applying the Fick's diffusion model, and effective moisture diffusion coefficients were calculated. A third order polynomial relationship was found to correlate the effective moisture diffusivity (D_{eff}) with moisture content. The effective moisture diffusivity increased with decrease in moisture content of carrot slices. Average effective moisture diffusivity increased from 6.33×10^{-9} to 1.14×10^{-8} m^2/s with increasing the microwave power.

Keywords : drying; modeling; moisture diffusivity; carrot slices, microwave power.

GJSFR-C Classification : FOR Code: 100505, 861401

USING OF SEMI-EMPIRICAL MODELS AND FICKS SECOND LOW FOR MATHEMATICAL MODELING OF MASS TRANSFER IN THIN LAYER DRYING OF CARROT SLICE

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Using of Semi-Empirical Models and Fick's Second Low for Mathematical Modeling of Mass Transfer in Thin Layer Drying of Carrot Slice

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Abstract - Drying behavior of carrot slices were studied at 200, 300, 400 and 500 W for constant sample thickness (5 mm) in a microwave dryer. By increasing the microwave output powers (200-500 W), the drying time decreased from 17.5 to 9.5 min. The drying process took place in the falling rate period. Six mathematical models for describing the thin-layer drying behavior of carrot slices were investigated. The models were compared based on their coefficient of determination (R2), root mean square error (RMSE) and reduced chi-square (X2) v alues between ex perimental and predicted moisture ratios. The results show that the Midilli model is the most appropriate model for drying behaviour of thin layer carrot slices. Moisture transfer from carrot slices was described by applying the Fick's diffusion model, and effective moisture diffusion coefficients were calculated. A third order polynomial relationship was found to correlate the effective moisture diffusivity (D_{eff}) with moisture content. The effective moisture diffusivity increased with decrease in moisture content of carrot slices. Average effective moisture diffusivity increased from 6.33×10^{-9} to 1.14×10^{-8} m^2/s with increasing the microwave power.

Keywords : drying; modeling; moisture diffusivity; carrot slices, microwave power.

I. Introduction

Drying of moist materials is a complicated process involving simultaneous heat and mass transfer. Mathematical modeling and simulation of drying curves under different conditions is important to obtain a better control of this unit operation and an overall improvement of the quality of the final product [1, 2, 3]. Currently, there are three types of thin-layer drying models used to describe the drying phenomenon of agriculture product, namely, theoretical model, which considers only the internal resistance to moisture transfer between product and heating air, semi-theoretical and empirical models which consider only the external resistance.

The drying process takes place in two stages. The first stage happens at the surface of the drying material at a constant drying rate and is similar to the vaporization of water into the ambient. The second stage drying process takes place with decreasing drying rate [4].

When drying process is controlled by the internal mass transfer, mainly in the falling rate period, modeling of drying is carried out through diffusion equations based on Fick's second law [4, 5, 6, 7]. Molecular diffusion is the main water transport mechanism and to predict the water transfer in food materials diffusion models based on Fick's second law are used.

Effective moisture diffusivity describes all possible mechanisms of moisture movement within the foods, such as liquid diffusion, vapour diffusion, surface diffusion, capillary flow and hydrodynamic flow. A knowledge of effective moisture diffusivity is necessary for designing and modeling mass-transfer processes such as dehydration, adsorption and desorption of moisture during storage. Researchers reported that effective moisture diffusivity increased with moisture content up to a limit value and then decreased and eventually became constant at high moisture contents [8, 9].

The aim of this research was the study and modelling of the drying kinetics of mass transfer during the microwave drying process of carrot slices. The effect of drying powers and moisture content on the effective moisture diffusivity of the dried slices was also studied.

II. Mateials and Methods

Carrot samples were procured from local vegetable market in Tehran, Iran. The samples were stored at 4±0.5 °C before they were used in experiments. The samples were removed from the refrigerator before experimentation and were allowed to attain room temperature. Carrots were washed under running water to remove the adhering impurities, and thinly sliced in thicknesses of 5 mm using a sharp stainless steel knife. The average initial moisture content of the samples were found to be 78.2 ± 0.7 % wet basis, as determined by using convective oven at 105 °C for 24h.

A domestic microwave oven (M945, Samsung Electronics Ins) with maximum output of 1000 W at 2450MHz was used for the drying experiments. The

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dimensions of the microwave cavity were 327×370×207 mm. The oven has a fan for air flow in drying chamber and cooling of magnetron. The moisture from drying chamber was removed with this fan by passing it through the openings on the right side of the oven wall to the outer atmosphere. The microwave dryer was operated by a control terminal which could control both microwave power level and emission time. Experiments were performed at four initial mass of 30 g at four microwave powers of 200, 300, 400 and 500 W. The moisture losses of samples were recorded at 15 s intervals during the drying process by a digital balance (GF-600, A & D, Japan) and an accuracy of ± 0.001 g.

For measuring the weight of the sample during experimentation, the tray with sample was taken out of the drying chamber, weighed on the digital top pan balance and placed back into the chamber. Drying was carried out until the final moisture content reaches to a level less than 10 % (w.b.).

The moisture ratio of samples during the thin layer drying experiments was calculated using the following equation:

$$MR = \frac{X_t - X_e}{X_0 - X_e} \quad (1)$$

where MR is the moisture ratio (dimensionless), Xt is the moisture content at drying time t (d.b.) and X0 is the initial moisture content (d.b.). The values of Xe are relatively small compared to Xt or X0. Thus, Eq. (1) can be reduced to MR=Xt/X0 [5].

The experimental sets of (MR, t) were fitted to six empirical models from the literature, using IBM SPSS Statistics 19. (SPSS, Inc.).

Newton model:

$$MR = \exp(-kt) \quad (2)$$

Henderson and Pabis model:

$$MR = a\exp(-kt) \quad (3)$$

Page's model:

$$MR = \exp(-kt^n) \quad (4)$$

Midilli model:

$$MR = a\exp(-kt^n) + bt \quad (5)$$

Wand and Singh model:

$$MR = 1 + bt + at^2 \quad (6)$$

Logarithmic model:

$$MR = a\exp(-kt) + b \quad (7)$$

Where k is the drying rate constant (1/min), and a, b and n are equation constants model.

The terms used to evaluate goodness of fit of the tested models to the experimental data were the coefficient of determination (R^2); root mean square error (RMSE) and the reduced chi-square (χ^2) between the experimental and predicted moisture ratio values. Statistical values are defined as follows:

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{N}(MR_{pre,i} - MR_{exp,i})^2}{\sum_{i=1}^{N}(MR_{pre,i} - \overline{MR}_{exp})^2}\right) \quad (8)$$

$$\chi^2 = \frac{\sum_{i=1}^{n}(MR_{exp,i} - MR_{pre,i})^2}{N - z} \quad (9)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(MR_{exp,i} - MR_{pre,i})^2}{N}} \quad (10)$$

In these equations, N is the number of observations, z is the number of constants, MRexp and MRpre are the experimental and predicted moisture ratios, respectively.

The drying rate of samples was calculated by using Eq. (11):

$$DR = \frac{X_{t+\Delta t} - X_t}{\Delta t} \quad (11)$$

where $X_{t+\Delta t}$ is moisture content at time t+Δt (d.b.), t is the time (min) and DR is the drying rate (d.b./min).

Fick's second equation of diffusion was used to calculate the effective diffusivity, considering a constant moisture diffusivity, infinite slab geometry and uniform initial moisture distribution:

$$MR = \frac{8}{\pi^2}\sum_{n=o}^{\infty}\frac{1}{(2n+1)}\exp\left(-\frac{(2n+1)\pi^2}{L^2}D_{eff}\,t\right) \quad (12)$$

where D_{eff} is the effective diffusivity (m^2/s), and L is the thickness (here half) of slab (m).

The Eq. (12) can be simplified by taking the first term of Eq. (13):

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff}\,t}{L^2}\right) \quad (13)$$

Eq. (13) is evaluated numerically for Fourier number, $F_0 = D_{eff} \times t/L^2$, for diffusion and can be rewritten as Eq. (14) can be rewritten as:

$$MR = \frac{8}{\pi^2}\exp(-\pi^2 F_0) \quad (14)$$

Thus:

$$F_0 = -0.101\ln(MR) - 0.0213 \quad (15)$$

The effective moisture diffusivity was calculated using Eq. (16) as:

$$D_{eff} = \frac{F_0}{\left(\frac{t}{L^2}\right)} \quad (16)$$

III. Results and Discussion

Fig. 1 shows the change in moisture ratio of carrot slices with time by microwave drying. A reduction in drying time occurred with increasing the microwave power level. On the other hand, mass transfer within the sample was more rapid during higher microwave power

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heating because more heat was generated within the sample creating a large vapor pressure difference between the centre and the surface of the product due to characteristic microwave volumetric heating. The time required for the lowering of moisture content of carrot slices to 0.1 from 3.59 on dry basis varied between 9.5 and 17.5 min depending on the microwave power level.

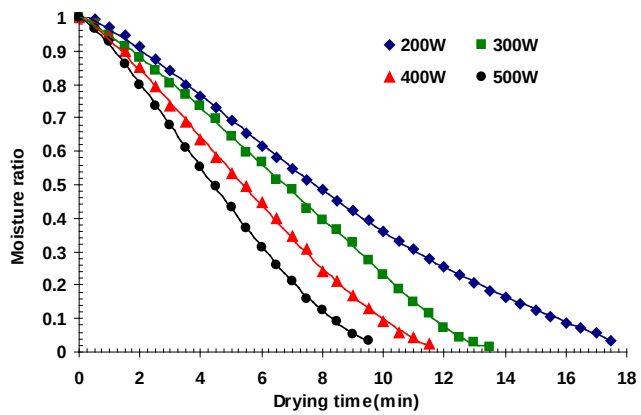

Figure 1 : Variation in moisture ratio as a function of drying time

The statistical results from models are summarized in Table 1. The values of mentioned tests were in the range of 0.7373–0.9999 for R^2, 0.00003–0.0665 for χ^2, 0.0054–0.2486 for RMSE. Based on the criteria of the highest R^2 and the lowest RMSE, and χ^2, the model of Midilli was selected as the most suitable model to represent the thin-layer drying behavior of carrot slices. Fig. 2 compares experimental data with those predicted with the Midilli model for carrot slices at 200, 300, 400 and 500W. The prediction using the model showed MR values banded along the straight line, which showed the suitability of these models in describing drying characteristics of carrot s lices.

Table 1 : Thin layer drying model constants and statistical parameters of sliced carrot

Model	P(W)	Constants	R^2	χ^2	RMSE
Newton	200	k=0.1313	0.8737	0.0125	0.1103
	300	k=0.1848	0.7373	0.0285	0.1656
	400	k=0.2153	0.8154	0.0211	0.1423
	500	k=0.2505	0.8477	0.0177	0.1266
Henderson and Pabis	200	k=0.1644; a=1.4794	0.9244	0.0182	0.1310
	300	k=0.2505; a=1.8251	0.8131	0.0665	0.2486
	400	k=0.2806; a=1.6672	0.8802	0.0428	0.1982
	500	k=0.319; a=1.5604	0.9047	0.0327	0.1674
Wang and Singh	200	a=0.0007; b=-0.0690	0.9945	0.0006	0.0228
	300	a=-0.0005; b=-0.0698	0.9969	0.0082	0.0875
	400	a=0.0005, b=-0.0951	0.9949	0.0006	0.0229
	500	a=0.0013; b=-0.1182	0.9946	0.0006	0.0228
Page	200	k=0.028; n=1.574	0.9980	0.0002	0.0145
	300	k=0.023; n=1.813	0.9903	0.0012	0.0327
	400	k=0.041; n=1.707	0.9940	0.0007	0.0248
	500	k=0.060; n=1.680	0.9961	0.0004	0.0193
Midilli	200	k=0.036; n=1.379; a=1.010; b=-0.007	0.9999	0.00003	0.0054
	300	k=0.024; n=1.519; a=0.994; b= -0.022	0.9999	0.0001	0.0094
	400	k=0.034; n=1.550; a=0.997; b=-0.020	0.9982	0.0007	0.0246
	500	k=0.063; n=1.492; a=0.996; b=-0.015	0.9999	0.0001	0.0064
Logarithmic	200	k=0.041; a=2.015; b=-0.961	0.9983	0.0002	0.0149
	300	k=0.015; a=5.931; b=-4.885	0.9964	0.0007	0.0250
	400	k=0.033; a=3.342; b=-2.298	0.9971	0.0003	0.0169
	500	k=0.047; a=2.888; b=-1.847	0.9973	0.0004	0.0179

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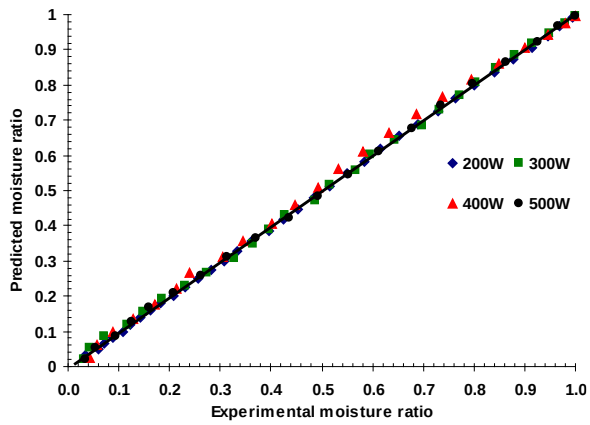

Figure 2 : Comparison of experimental moisture ratio with predicted moisture ratio from the Midilli model for carrot slices

Fig. 3 shows the changes in drying rate as a function of drying time at the different powers. It is clear that the moisture content and drying rate decrease continuously with drying time. The drying rate was rapid during the initial period but it became very slow at the last stages during the drying process. The moisture content of the material was very high during the initial phase of the drying which resulted in a higher absorption of microwave power and higher drying rates due to the higher moisture diffusion. As the drying progressed, the loss of moisture in the product caused a decrease in the absorption of microwave power and resulted in a fall in the drying rate. Similar results have been observed in the drying of different fruits and vegetables: kiwifruit [10]; hazelnut [11]; carrot pomace [12]; amelia mango [13]; pineapple, mango, guava and papaya [14] and apple [6]. Constant drying rate period was not observed during the drying of carrot slices. This shows that diffusion in dominant physical mechanism governing moisture movement in the samples.

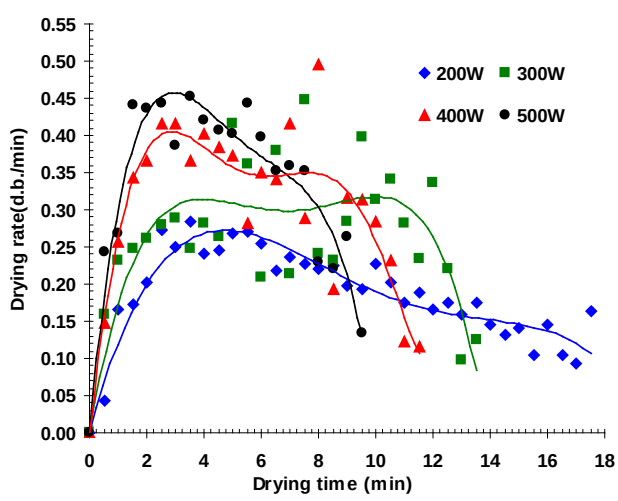

Figure 3 : Drying rates for carrot slices at different microwave powers

Variation in effective moisture diffusivity of carrot slices with moisture content at different microwave power levels is shown in Fig. 4. The effective moisture diffusivity increased with decrease in moisture content. However, the moisture diffusivity further was higher at any level of moisture content at higher microwave power level, resulting into shorter drying time. This may indicate that as moisture content decreased, the permeability to vapour increased, provided the pore structure remained open. The temperature of the product rises rapidly in the initial stages of drying, due to more absorption of microwave heat, as the product has a high loss factor at higher moisture content. This increases the water vapour pressure inside the pores and results in pressure induced opening of pores. In the first stage of drying, liquid diffusion of moisture could be the main mechanism of moisture transport. As drying progressed further, vapour diffusion could have been the dominant mode of moisture diffusion in the latter part of drying. Sharma and Prasad [8]; Sharma et al. [9] also reported similar trend in the variation in the moisture diffusivity with moisture content.

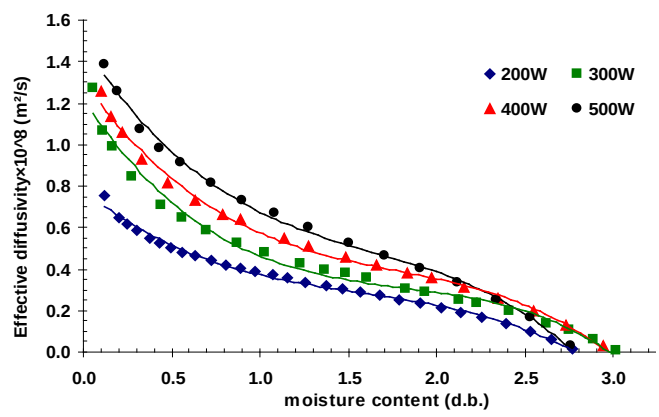

Figure 4 : Variation in effective moisture diffusivity with moisture content at different microwave powers

A third order polynomial relationship was found to correlate the effective moisture diffusivity with corresponding moisture content of carrot slices and is given by Eq. (17)

$$D_{eff} = (A + BX + CX^2 + DX^3) \times 10^{-8} \qquad (17)$$

where A, B, C, D is the constants of regression, and X is moisture content (d.b.)

Regression constants for microwave drying of carrot slices under different powers are presented in Table 2. The high values of R^2 are indicative of good fitness of empirical relationship to represent the variation in effective moisture diffusivity with moisture content of carrot slices.

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Table 2 : Regression coefficients of effective moisture diffusivity for different microwave powers

P(W)	A	B	C	D	R^2
500	1.4877	-1.3507	0.6745	-0.1372	0.9962
400	1.3081	-1.2129	0.5893	-0.1109	0.9955
300	1.2130	-1.2750	0.6410	-0.1174	0.9862
200	0.7791	-0.6854	0.3530	-0.0745	0.9944

The average effective moisture diffusivity was calculated by taking the arithmetic mean of the effective moisture diffusivities that were estimated at various levels of moisture contents during the course of drying, as shown in Fig 5. Average values of effective diffusivity for different microwave power are presented in Table 3. The D_{eff} values of the carrot slices were within the general ranges of 10^{-9} to 10^{-11} m^2/s for biological materials [6, 12, 15]. The values of D_{eff} are comparable with the reported values of 1.0465×10^{-8} to 9.1537×10^{-8} m^2/s mentioned for apple pomace microwave drying [6], 1.14×10^{-6} to 6.09×10^{-6} m^2/s for tomato pomace microwave drying at 160-800W [16], 0.55×10^{-7} to 3.5×10^{-7} m^2/s for Gundelia tournefortii microwave drying at 90-800W [7].

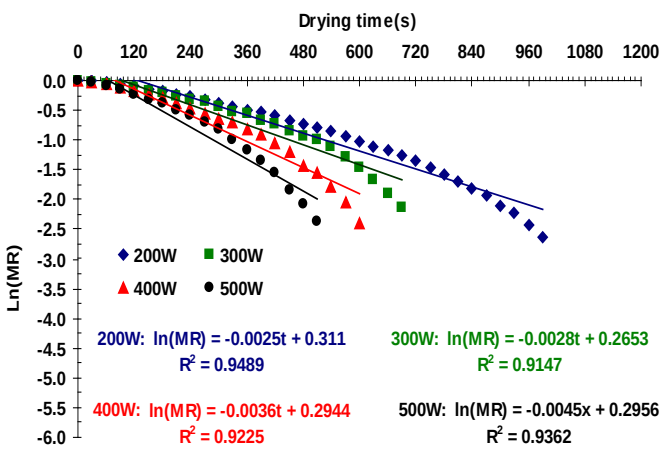

Figure 5 : Variation in ln (MR) and drying time (in seconds) for carrot slices dried at different microwave powers

Table 3 : Result of average effective diffusivity of carrot slices with different microwave power levels

P(W)	Average effective diffusivity (m^2/s)
500	1.14×10^{-8}
400	9.12×10^{-9}
300	7.09×10^{-9}
200	6.33×10^{-9}

A linear regression analysis on the average diffusion coefficient with microwave power resulted in the following relationships:

$$(D_{eff})_{average} = 4\times10^{-9}\exp(0.002P) \quad R^2 = 0.9788 \quad (18)$$

where P is the microwave power (W).

IV. Conclusion

In this study, microwave drying characteristics of carrot slices were investigated. The drying curves of carrot slices did not show a constant rate drying period. The drying took place in the falling rate period during drying process. To explain the drying characteristics of strained yoghurt, nine drying models were applied; however, the model developed by Midilli model showed good agreement with the experimental data. The effective diffusivity was computed from Fick's second law, the values of which varied between 6.33×10^{-9} to 1.14×10^{-8} m^2/s, over the microwave power level range. The effective diffusivity increases microwave power level increases. The effective moisture diffusivity increased with decrease in moisture content of carrot slices. A third order polynomial relationship existed between effective moisture diffusivity and the moisture content of carrot slices.

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- Two Column with Equal Column with of 3.38 and Gaping of .2
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- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
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Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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