INTERNATIONAL JOURNAL OF ENVIRONMENT

Volume-9, Issue-1, 2019/20 ISSN 2091-2854

Received:17 November 2019 *Revised:28 January 2020* *Accepted:30 January 2020*

ROLE OF INTRA-SPECIFIC VARIETAL MIXTURE IN POLLINATION SERVICES: A CASE STUDY IN BUCKWHEAT (*Fagopyrum esculentum* Moench)

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Abstract

Buckwheat is one of the crops on which pollination services from honeybees and other insect pollinators are necessary for obtaining quality and higher grain yield. Increasing plant biodiversity is an important strategy to attract and restore pollinators in an agro-ecosystem. Among many ways to increase plant biodiversity, the present study aimed to identify whether the increasing intra-specific crop diversity enhances pollinator diversity in buckwheat (*Fagopyrum esculentum* Moench). The field experiment was conducted at research field of Hill Crop Research Program, Kabre, Dolakha district, Nepal at an altitude of 1750 m asl during 2016. Thirteen treatments, each representing either a single or combination of two or three buckwheat genotypes were laid out in randomized complete block design (RCBD) with two replications. Pollination diversity or richness as well as their foraging behavior in the buckwheat flowers and associated yield parameters were measured for each treatment. Plots with higher intra-specific varietal mixture were found to attract more pollinators and had higher yield than plots with two or single genotypes. Pollinator visitation was higher during morning hour of the day (9 am to 10 am), which was followed by mid-day (12 noon to 1 pm) and the least visitation was observed during late afternoon (3 pm to 4 pm). The benefit of increasing intra-specific crop diversity on pollination services is not optimized and practiced under current farming practices. Hence, multi-location and multi-season studies are recommended to strengthen the current findings for future endeavors.

Keywords: Agroecosystem; Biodiversity; Buckwheat; Conservation biology; Honeybees

DOI: http://dx.doi.org/10.3126/ije.v9i1.27651

Introduction

Animal pollination is vital for maintaining biodiversity and increasing crop yield. More than 800 cultivated crops, covering 75% crop species, depend on animal pollination at least to some degree (Nicholls and Altieri, 2013). Animal pollination alone accounts for 35% of global production (Bartomeus et al., 2014). Despite its important role in global crop production, the increasing anthropogenic activities, for example, habitat destruction due to monoculture, change in land use, etc. has caused a decline in bees and other insect pollinators. Therefore, improving pollinator-friendly habitats to increase pollinators' services is a critical issue (Nicholls and Altieri, 2013). Restoring plant biodiversity within and around crop fields could restore pollinator-friendly habitats for bees and other insect pollinators and therefore increase pollination services in an agro-ecosystem and offer a resilient production system (Nicholls and Altieri, 2013; Joshi et al., 2019). One of the ways to establish a pollinator-friendly environment is through increasing intra-specific crop diversity. This kind of study is important for those crops that depend on insects for pollination and where pollinator habitats are declining. Buckwheat is one good example due to its entomophily nature for pollination.

Buckwheat (*Fagopyrum esculentum* Moench) is an indeterminate 'Pseudocereal' belonging to family Polygonaceae (Jacquemart et al., 2012). The crop is cross-pollinated and entomophily; it is said that buckwheat pollen is not windblown, therefore insect pollination is required (Rajbhandari, 2010). Buckwheat pollination is determined by many factors, such as pollinator characteristics, floral morphology, physiology, habitat richness, weather conditions, etc. (Huang and Giray, 2012; Aryal et al., 2016). Honeybees are the major group of pollinators, which increase buckwheat productivity by 25-30% (Cawoy et al., 2009; Rajbhandari, 2010; Aryal et al., 2016). Despite the huge importance of bees and other natural pollinating agents, the importance of pollination is still undermined. Under such situation, restoring the pollinator population in agro-ecosystems is imperative. Pollinators can be potentially restored through increasing plant diversity. Among many ways to increase plant diversity, using an intra-specific varietal mixture of crops has been considered as an important strategy, however there is limited literature available in the context of buckwheat. Therefore, the role of the intra-specific varietal mixture on the diversity/abundance and foraging behavior of pollinators along with yield parameters were studied using on-farm field measurement as a baseline study for future endeavors.

Materials and Methods

An on-farm experiment was conducted at a research field of Hill Crop Research Program (HCRP), NARC, Kabre, Dolakha district at an altitude of 1750 m asl, representing the mid-hill region. The climate is characterized by warm temperate with limited rainfall. The soil pH ranged from 4.5-6.5 and the organic carbon is very low (NARC, 2018). Seeds of five genotypes such as ACC#5670, ACC#2213, ACC#6529, ACC#2234 and IR-13 were collected from National Agriculture Genetic Resources Centre (NAGRC- Nepal Genebank). These genotypes were allocated either singly or in mixture of two or three buckwheat genotypes depending upon agro-morphological characteristics and used as treatments. In total, the present study contains 13 treatments where five treatments constituted a single genotype, four treatments constituted a combination of two intraspecific genotypes and four treatments constituted a combination of three intraspecific genotypes (Table 1). The treatments were laid out in randomized complete block design (RCBD) with two replications. The plot size was 4 square meters (2-m x 2-m). Compost was applied at 10 Mt/ha before 15 days of final land preparation. Chemical fertilizer was applied manually at 30:30:0 NPK kg/ha during final land preparation. The seeds were sown in a continuous row, and row to row distance was 25 cm. The other agronomic practices were adopted according to HCRP general guidelines (NARC, 2018).

Table 1: Treatment details

Treatments	Treatment detail (mixture components)	Remarks
T1	V1=ACC#5670	Collected from Julsale, Solukhumbu district at an altitude of 2865 m asl during 1986, days to flowering – 43, days to maturity – 81, plant height – 81 cm
T2	V2=ACC#2213	Collected from Laban, Dhading district at an altitude of 580 m asl during 1989, days to flowering – 45, days to maturity – 81, plant height – 81 cm
T3	V3=ACC#6529	Collected from Kabre, Dolakha district at an altitude of 1750 m asl during 1986, days to flowering – 46, days to maturity – 80, plant height – 80 cm
T4	V4=ACC#2234	Collected from Ratanchura, Sindhuli district at an altitude of 1350 m asl during 1989, days to flowering – 44, days to maturity – 83, plant height – 83 cm
T5	V5=IR-13	Accession NGRC-05051, Origin-Japan, intermediate growth habit, white flower, days to flowering-30, days to maturity-72, plant height-80 cm (Joshi *et al.*, 2017).
T6	V1+V2	
T7	V4+V3	
T8	V2+V4	
T9	V4+V5	
T10	V2+V3+V4	
T11	V1+V2+V3	
T12	V3+V4+V5	
T13	V2+V4+V5	

V1 = first variety chosen in the present study, V2 = second variety chosen in the present study and so on, ACC# represents accession number given by Agriculture Botany Division, NARC which was used to conserving local plant genetic resources before the establishment of National Agriculture Genetic Resources Centre (NAGRC-Nepal Genebank).

Observation on diversity and abundance and foraging behavior started from 25% flowering until flowering period during the winter season (7 to 24 November) of 2016, using specific observation methods, as described below.

Pollinator diversity and abundance

Diversity and abundance of pollinators' population were recorded throughout the flowering period in three-days intervals. In total six observations (9 November, 12 November, 15 November, 18 November, 21

November and 24 November) were made. Yellow pan-trap and sight-count methods were used to study pollinators diversity, as described below.

Yellow pan-trap method: Each ultra-violet painted yellow colored pan-trap (17 cm diameter and 9 cm deep) with detergent water is placed at the center of each plot. The observations were recorded on three consecutive days. The pollinators or specimens were collected from pan trap during the morning period and kept in glass vials with 70% ethyl alcohol. These glass vials with pollinators were then taken to Taxonomy Laboratory of Entomology Division, NARC, Khumaltar, Lalitpur for further identification.

Sight-count method: An instant visual observation of pollinators was done for each treatment. Five plants were selected randomly and marked from each treatment. The pollinators visiting the floral parts of the plant were recorded for one minute. Observations were made at three different hours of the day (9-10 am, 12-1 pm and 3-4 pm) to determine abundance and peak foraging hours of the day for pollinators.

Foraging behaviour of the pollinators

The time of initiation and cessation of foraging (hours of the day) of honeybees for each observation date was recorded using sight-count method. Also, the total duration of foraging activity (in hours) and time spent on the inflorescence/flowers (in seconds) during each observation day were recorded. To calculate the time spent on the inflorescence/flowers (in seconds), observation was started at 10 am during each observation day.

Measuring yield parameters

Yield (kg) per plot was determined at 12% grain moisture level, which then converted to yield (kg) per hectare. Also, thousand kernel weight (g) was determined for each plot.

Data processing and analysis

The data obtained from the aforementioned activities were entered into Microsoft Office Excel 2007. The treatment differences in terms of pollination diversity and abundance and best foraging time were compared using analysis of variance (ANOVA) using GEN-STAT 18th edition. The means comparison was done using Fisher's LSD test.

Results and discussion

Pollinator abundance in different buckwheat genotypes treatments

The flower of buckwheat attracts many pollinators. The average number of pollinators trapped in yellow pan trap showed the highest abundance (11.77±2.28 pollinators) during the second observation date (12 November), and the lowest (7.46±2.01 pollinator) in last observation date (24 November), which correlates with less flowering during this time. The average number of pollinators visit was observed highest in treatment T12 or V12 (12.58±1.77 pollinators) while it was found lowest in treatment T3 or V3 (7.33±1.94 number of pollinators). The ANOVA results, in general, show that the number of pollinators was significantly higher in the mixture of three buckwheat genotypes than the mixture of two or no mix (single) of genotypes (Figure 1). However, treatment T5 (V5 = IR-13) attracted a higher number of pollinators. IR-13, a sweet type, had early flowering and more flower density than other treatments. Plots with higher flower density offer more nectar and pollen sources, therefore, attract more pollinators.

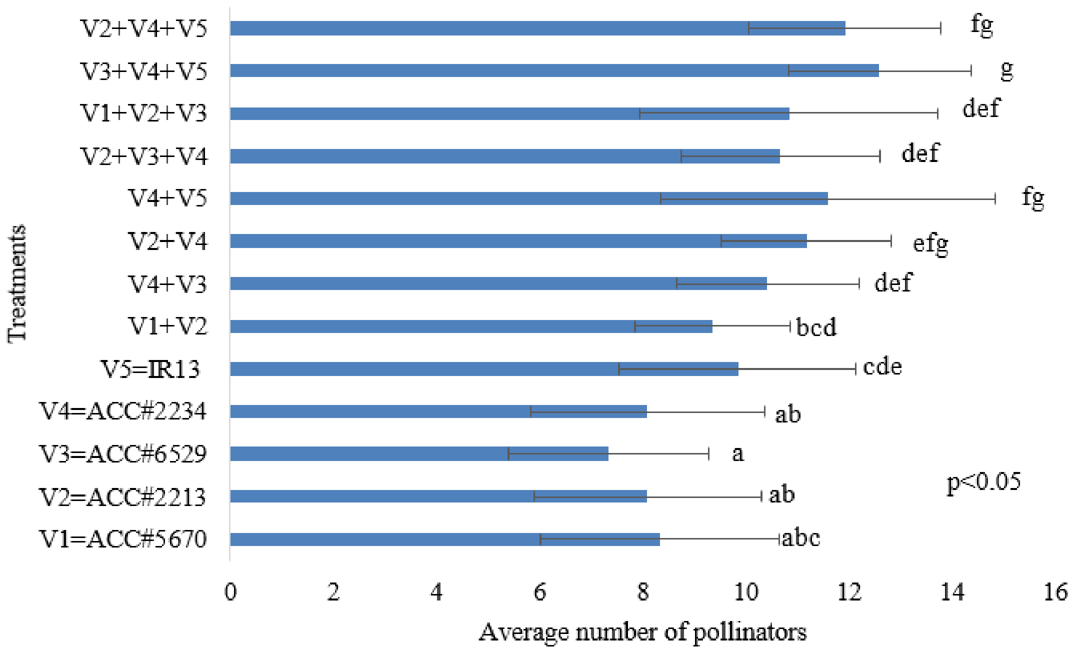

Figure 1: Pollinators population abundance of different buckwheat genotypes/mixtures in yellow pan trap at Kabre, Dolakha district, 2016. Means followed by the same alphabet do not differ significantly by Fisher's LSD test at $p<0.05$. The bar represents the standard deviation of the respective treatment.

Pollinators population visit in different buckwheat genotypes treatments

The pollinators' visit was observed more (2.18±1.07) during first observation date (9th November) followed by third observation date (15th November, 2.04±0.52) while it was found least during fifth observation date (21st November, 1.60±0.68). This might be due to less flowers available during later days. The pooled data revealed that significantly ($p<0.01$) highest number of pollinators were observed during 9 to 10 am of the day (2.38±0.42 pollinators), followed by 12 noon to 1 pm (1.99±0.59 pollinators) and 3 pm to 4 pm (1.30±0.45 pollinators) (Figure 2). Singh et al. (2008) reported morning hour (8:30-9:30 am) as the peak foraging period in buckwheat followed by afternoon period for the Himalayan honeybee, *Apis cerana* F., however, the authors argued both periods as very important from pollination perspectives. Also, the high-altitude buckwheat cultivation areas are generally characterized by good sunshine during the morning, and a windy cold climate after 12 pm. Pollinators need minimum ambient temperature for foraging purposes. Moreover, recent research reports reveal the threat to pollinators due to use of pesticides has become a serious problem (Potts, et al., 2010; Abrol, 2012; Gebremedhn et al., 2014; Chagnon et al., 2015; Botías and Sánchez-Bayo, 2018). Therefore, the pollinators could be protected from chemical pesticide hazards by not spraying pesticides at least during the morning and mid-day hour when foraging activity of pollinators is found to be highest. In a different way, spray can be done in the later hour of the day, which is not an attractive hour for the bees (Gebremedhn et al., 2014).

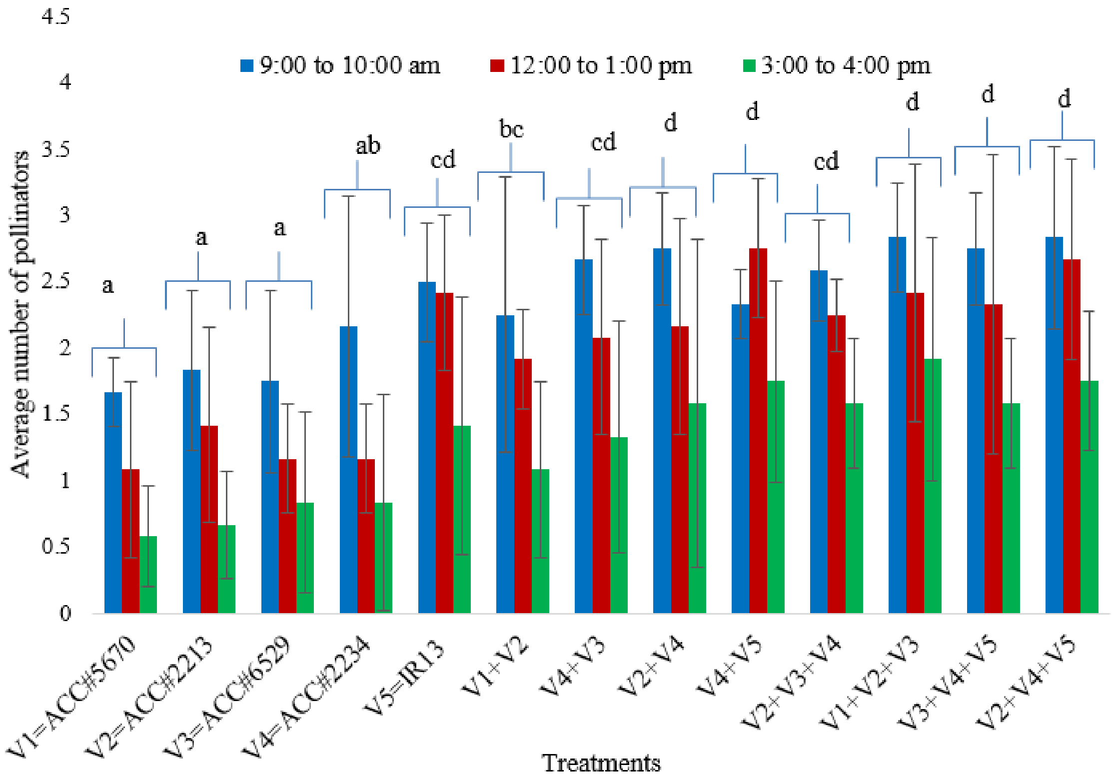

Figure 2: Average number of pollinators' visit in different buckwheat plots with single or mixed genotypes during different observation days and hours of the day at Kabre, Dolakha district, 2016. The time factor showed a significant difference for each treatment or varietal mixture and therefore multiple comparisons are not shown in the figure. Means of the varietal mixture based on pooled data followed by the same alphabet do not differ significantly by Fisher's LSD test at $p<0.05$. The bar represents the standard deviation of the respective treatment.

Among the different treatments, the number of pollinators' visit was comparatively less in plots with a single genotype than with mixture, in general (Figure 2). Though not significantly different the number of the pollinators' visit was more in treatment T13 (2.42±0.35) with a mixture of three genotypes. Again, as found in yellow trap, treatment T5 (IR-13) had more pollinators than the others.

Initiation and cessation time of foraging by pollinator in different buckwheat genotypes treatments

The earliest initiation of foraging by pollinator started from 7:25 am to 8:00 am. Similarly, the cessation time of pollinator visit ranged from 4:45 pm to 5:10 pm. The total foraging hours of the pollinator varied from 8.45 to 9.45 hours. Singh et al. (2008) reported the 10-hour duration of foraging activity in buckwheat crop observed at Kirtipur, Kathmandu, Nepal during November month of the study. Aryal et al. (2016) and Campbell et al. (2016) found a huge number of pollinating insects were attracted by buckwheat flowers, along with a huge diversity of beneficial predators and parasitoids. These useful traits of buckwheat show the possibility of using buckwheat as a cover crop to manage other insect pests or to augment pollinators to obtain higher yield through better plant protection and pollination services.

At the present study, the correlations between meteorological information such as minimum temperature, maximum temperature, relative humidity to foraging behavior parameters including total foraging hours of the pollinators were not much related. This might be due to similar pattern of meteorological information during the study period (Annex I). Although there were no significant relationships, the foraging activity of the pollinators was related to the existing temperature: the lower the temperature, the less foraging time for pollinators.

Time spent by honeybees in buckwheat flower

The time spent by the honeybees in the inflorescence of buckwheat flowers ranged from 2.0 to 11.0 seconds, but this was not significantly different with treatments. In general, time spent by honeybees in mixed buckwheat genotypes was comparatively higher than in single genotype (except IR-13) (Figure 3).

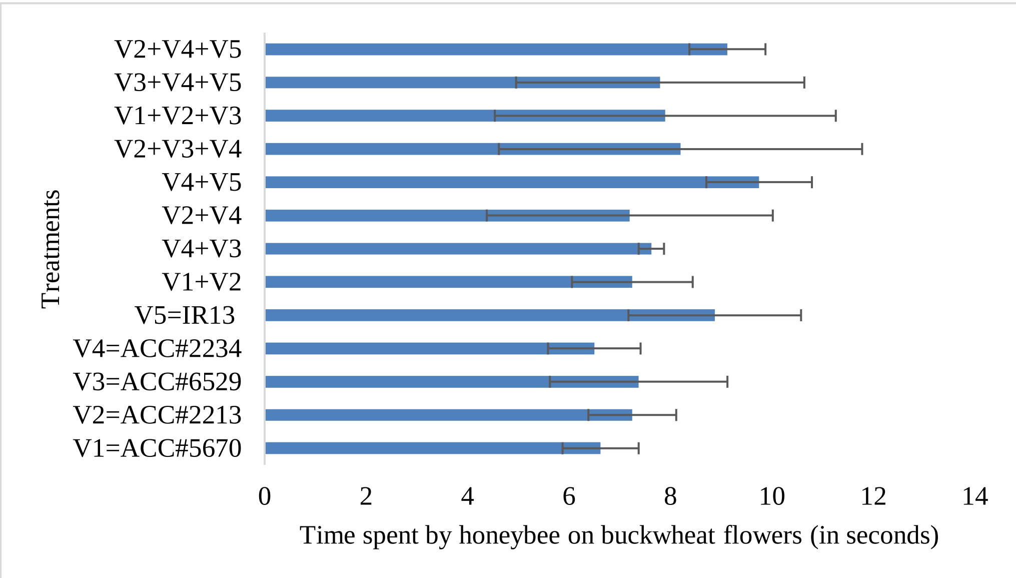

Figure 3: Time spent (seconds) by honeybees on buckwheat flowers in different mixture combinations at Kabre, Dolakha district, 2016. The bar represents the standard deviation of the respective treatment.

Effect of buckwheat genotypes treatments on yield parameters

Most of the plots with higher intra-specific buckwheat diversity performed better than others, resulting in higher grain yield. Treatment T13 (V2+V4+V5) significantly obtained the highest yield (912.50 kg/ha) compared to most of the other treatments ($p<0.05$) (Table 2). The increased yield could be attributed to more frequent visits for plots with mixed genotypes (Figure 1 and 2). Again, IR-13 yielded significantly higher grain yield (805 kg/ha) even compared to some mixture plots (Table 2). This result is in line with the fact that IR-13 attracted more pollinators than some other plots with different mixtures of buckwheat genotypes (Figure 1 and 2), which increased the chance of seed set and resulted in higher grain yield. Also, the genotype might have higher genetic yield potential than the others. There were no significant differences between treatments in terms of thousand kernels weight (g), this may be due to selecting only robust kernels during sampling.

In general, plots with higher intra-specific varietal mixture attracted more pollinators. It is possible that a varietal mixture offers food material for pollinators for a longer period of time, as initiation and cessation of flowering might not be the same for all buckwheat varieties grown in the field. Also, crop diversity might provide a wide range of diversity of plants in terms of flower color, stem color that provides cues to attract pollinators. Future research should seek the specific traits of plant that attracts pollinators when they are grown in a mixture or in single genotype.

Table 2: Effect of intra-specific buckwheat diversity on yield parameters at Kabre, Dolakha district, 2016

Treatments	Grain yield (kg/ha)	Thousand kernel weight (g)
T1 (V1=ACC#5670)	687.50	24.67
T2 (V2=ACC#2213)	797.50	24.6
T3 (V3=ACC#6529)	755.00	23.79
T4 (V4=ACC#2234)	725.00	24.88
T5 (V5=IR13)	805.00	27.15
T6 (V1+V2)	667.50	23.74
T7 (V4+V3)	850.00	26.44
T8 (V2+V4)	680.00	24.03
T9 (V4+V5)	890.00	22.34
T10 (V2+V3+V4)	725.00	25.09
T11 (V1+V2+V3)	807.50	25.79
T12 (V3+V4+V5)	837.50	24.34
T13 (V2+V4+V5)	912.50	25.96
Grand mean	780.00	24.83
CV %	13.1	10
LSD (0.05)	52.49	ns
P-Value	<.001	0.855

ns: means not significant at level 0.05 of probability.

Conclusions

The buckwheat varietal mixture or crop population with high intra-specific crop diversity was found to attract more pollinators than the plots with single or few genotypes. Varietal mixture plot also had higher grain yield. Plants attract more honeybees and other insect pollinators in the morning hour than mid-day and late afternoon of the day. This fact may be implacable at high-hill plantations in relation to weather parameters, as wind velocity will be more during noon and evening hours. This statement could provide a guideline for applying pesticides without harming pollinators, and also support a release of pollinators in deficient areas. As this research result is based on a single cropping season on-farm experiment, conducting multi-season trials at multi-locations would strengthen these findings.

Acknowledgment

The authors are grateful to UNEP/GEF supported project Integrating Traditional Crop Genetic Diversity into Technology: Using a Biodiversity Portfolio Approach to Buffer against Unpredictable Environmental Change in the Nepal Himalayas (Local Crop Project). The project is coordinated by the Bioversity International in collaboration with Nepal Agricultural Research Council (NARC), Department of Agriculture (DoA) and Local Initiatives for Biodiversity, Research and Development (LI-BIRD). We appreciate Dr. Hira Kaji Manandhar and four anonymous reviewers for their feedback on the draft report.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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Annex I. Weather report during buckwheat flowering time (November 2016)

Date	RH at morning	RH Evening	Max Temp at Morning	Min Temp at Morning	Max Temp at Evening	Min Temp at Evening
1-Nov	65	57	22.5	8	23	17
2-Nov	65	64	23	9.5	23	17
3-Nov	64	64	24	7.5	22.6	16.2
4-Nov	57	64	23	6	23	17
5-Nov	57	64	23	7	23	18
6-Nov	57	71	21	6	21.5	15
7-Nov	64	80	21.5	8.5	21	14
8-Nov	64	80	21.5	8	21.4	15
9-Nov	64	73	21	7	21.5	16.6
10-Nov	64	73	21.5	8.5	22	17
11-Nov	73	73	22	11.5	22	17
12-Nov	65	81	22	10	22.4	18.5
13-Nov	72	81	22	9	22.4	18.5
14-Nov	70	72	22	6	22	17
15-Nov	47	62	22	7	21	14
16-Nov	45	72	21.5	6	22	17
17-Nov	63	62	20	5.5	20	15
18-Nov	55	72	20	6	20	15.5
19-Nov	62	79	20	6	19	13
20-Nov	71	71	19	7	20	14
21-Nov	63	72	20	7	21	16
22-Nov	62	71	21	7.5	20	14
23-Nov	71	71	21	8	20	15
24-Nov	71	71	20	7.5	20	15
25-Nov	71	62	20	6.5	19	14
26-Nov	62	68	19	8	20.5	14.5
27-Nov	62	71	20.5	9	20	15
28-Nov	55	80	19.5	9	18.5	13.5
29-Nov	63	71	19	7.5	20	15
30-Nov	62	61	20	7.5	19	13

RH = Relative humidity