

Spraying Bees with Sugar Syrup to Control *Varroa* Mites with Less Passive Impacts

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Abstract

Bees (*Apis mellifera*) are very valuable to agriculture. Bees are the main target of many pests and parasites, including mites. These mites can cause serious damage to bees. Therefore, *varroa* mites need to be controlled. Sugar syrup alone or with larva extract or propolis extract were used for comparative testing. According to the results, spraying bees with syrup can help control the passive effects of *varroa* mites on bees compared to propolis extracts and drone larvae extracts. Because this parameter is relatively stable during the process, winter is considered to be an assessment of bee grooming behavior.

Keywords

Honey bees; Grooming behavior; Sugar syrup; Drone larvae; Propolis.

Introduction

Honey bees, *Apis mellifera*, are very valuable to the agricultural sector. They are the main pollinators to various plants and beekeeping is considered as source of income to many people. Honey bees are the main target to many pests and parasites including *Varroa* mites. These mites can cause severe damages to the bees. Honey bees can protect their colonies from *Varroa* mites using specific behaviors including grooming behavior (GB). Causing damages to *Varroa* mites (i.e. GB) is a heritable character in *A. mellifera*. Phoretic *Varroa* mites; foundress, gravid or daughter mites are exposed

to grooming by bees especially daughter mites. The GB includes self-grooming and social grooming which comprises of groomers and recipient bees (Abdelghany et al. 2017; Abdella et al. 2016; Abdel-Naby et al. 2016). The GB can be stimulated using some safe materials including inert sugar. Other safe method to control *Varroa* includes sprinkling bees with sugar syrup. Using sugar syrup as a spray over bees has been found to be less harmful to bees than sugar dusting. *Varroa* mites attract to nurse bees, forager bees or even larvae at certain ages based on various factors including the reproductive stage of the mites, and distance from open brood cells. It is known that *Varroa* mites attract to drone

cells more than worker cells. Thus, it is hypothesized that using drone larvae extract mixed with sugar syrup as a spray over bees can disturb *Varroa* mites and enhance the GB. Propolis extract is another material that can be mixed with sugar syrup to control *Varroa*. Propolis extracts have shown narcotic and lethal effects on *Varroa* mites (Ahmad et al. 2017; Ahmad et al. 2016; Ajaib et al. 2016).

The GB is differed among bee species, subspecies and hybrids. GB can be evaluated under field conditions by calculating the percentage of damaged mites from the total number of fallen mites. A laboratory assay has been developed by Aumeier (2001) to assess the GB of honey bees artificially infested with *Varroa* mites. Fluctuations have been found in *Varroa* populations over months. In fact, the stability degree of the GB within the same bee colonies in the course of time especially during winter period has not been fully studied. It is known that brood rearing activity is very low during autumn and winter. Also, the longevity of winter bees is high. Thus, it is expected that adult bee populations in the colonies are greatly stable during winter. Therefore, studying the GB of the same group of bees is possible (Aleku et al. 2017; Ali et al. 2017).

There are various species of *Varroa* mites but *Varroa destructor* is the one causing damages to *A. mellifera*. This particular species is common in various parts of the world including Egypt. This species can be differentiated than other *Varroa* species by measuring body length and width to calculate ratio of body size. There are approximately 15 haplotypes of *V. destructor*. It is possible to identify four morphotypes of *Varroa* mites using morphometric characterization. So far, it is not completely known if the body morphology can be fluctuated (i.e. increased or decreased) within the same population of *Varroa* over time. A study in Ukraine has shown variations between morphological characteristics of summer and winter *Varroa* mites. Still, the fluctuations in morphological characteristics, mainly body length and width, need more investigations especially during winter. During this season foraging activity of honey bees is very low due to low air temperature and rains (Farooqi et al. 2017). Thus, the transportation of new *Varroa* mites by forager bees to their colonies is not highly expected. Also, it is anti-

ciated that *Varroa* population are stable during winter. In this study, sugar syrup was used alone or as mixture with drone larvae extract or propolis extract to control *Varroa* mites and to evaluate their impacts on GB and honey bees. Moreover, fluctuations in GB and *Varroa* morphology were studied during winter (Ali and Nawaz 2017, 2016; Amin et al. 2017).

Materials and Methods

Honey Bee Colonies

The colonies were located at an apiary at Damanhour city, Egypt. Each colony was provided with a lower drawer to facility the collection of fallen *Varroa* mites. Wire meshes were used to separate the beehive bodies than the drawers. Therefore, any fallen mites cannot be attack again by the bees. All the colonies were hybrids of Carniolan honey bees.

Effects of Sugar Syrup (SS), Extracts of Drone Larvae (EDL) and Propolis (EP) on *Varroa* Mites and Honey Bees

Effects of three safe materials on the grooming behavior of honey bees were evaluated during spring 2017: (I) sugar syrup (SS) 1:1 (suagr:water, w/w) (using 4 mL per comb), (II) sugar syrup 1:1 mixed with drone larvae extract (EDL) (20 drone larvae at 5th day were dissolved in 100 mL water and filtered, then 2 mL of the extract was mixed with 2 mL sugar syrup per comb), and (III) sugar syrup 1:1 mixed with propolis extract (EP) (5 gm propolis was mixed with 100 mL water and filtered, then 2 mL of the extract was mixed with 2 mL sugar syrup per comb). Four Carniolan hybrid colonies were used per each treatment. The colonies had approximately the same strength with 5 combs covered with bees. The fallen mites over 11 days were collected directly prior the treatments. Then, the cumulative fallen mites after the treatment period (each treatment was repeated three times with 4 days interval with a total period of 11 days) were collected directly. The grooming behavior of the colonies was assessed as percentage of groomed mites from the total number of collected mites. The percentages of groomed mites were compared after and before treatments.

The effects of these materials on nurse bee workers

were assessed under laboratory conditions. Each of SS, EDL, and EP were replicated four times (four jars and 15 bees per jar, a total of 60 bees per treatment). The jars were covered with mesh covers. The treatments were presented daily to the bees using cotton pieces saturated with each treatment above the mesh covers. The number of dead bees was counted daily for 7 days. Then, the mortality rates were calculated in each jar by dividing the daily number of dead bees on the total number of bees per jar (15) x100. Means were then calculated and compared. Fluctuations in the grooming behavior

This experiment and the next one were done using 6 colonies. Number of *Varroa* mites collected from these colonies was counted weekly from November 2016 until February 2017. The mites were classified as normal or deformed (groomed) using a light microscope. The mites with body malformations (i.e. incomplete chelicera, legs and/or shield) were considered as deformed. Then, percentage of groomed *Varroa* was calculated by dividing the number of groomed *Varroa* on the total number of *Varroa* X 100. The percentages of groomed *Varroa* were then compared over the experimental period.

Fluctuations in Varroa Morphology

The fallen *Varroa* mites were collected from the six colonies over the period from November 2016 until February 2017. The lengths and widths of *Varroa* were measured weekly. Only *Varroa* mites with complete bodies were considered to obtain correct widths and lengths while those with deformed body shields were not. The mites were scanned using scanner (Canon, k10352, LiDE 110, Vietnam) at a high resolution of 1200 dpi to obtain clear images. The lengths and widths were subsequently measured using computer program (ScanPhoto method). The measurements were compared over the study period to detect any morphological fluctuations. Also, the body ration (=body width/body length) was calculated.

Statistical Analysis

The comparison between groups was done using ANOVA followed by Post Hoc using Duncan's Multiple Range test. Also, t-test was used to compare percentage of groomed *Varroa* mites before and after the treatments.

Each of degree of freedom (DF), F value and P value were presented. The variations were considered significant when $P \leq .05$. The percentages were transferred using arcsine transformation before the analysis. For percentage of dead bees, Kaplan-Meier test was used to calculate the estimated survival means of the groups. Then, the significant differences between groups were determined using Log Rank (Mantel-Cox test). The data were analyzed using SAS v. 9.1.3 and SPSS v. 16.

Results

Effects of Sugar Syrup (SS), Extracts of Drone Larvae (EDL) and Propolis (EP) on Varroa Mites and Honey Bees

The mean number of fallen mites as difference between after and before the treatments was high to EP with 10.50 ± 2.06 mites, followed by EDL with 8.50 ± 3.71 mites and finally SS alone with 5.25 ± 2.21 mites. However, neither EP nor EDL were significantly different than SS (DF = 2, F = 0.92, P = .43 > .05). The mean percentage of groomed mites before treatments was 0.33 ± 0.04 , 0.38 ± 0.06 , 0.39 ± 0.06 mites to SS, EDL, and EP, respectively. The mean percentage of groomed mites after treatments was 0.64 ± 0.12 , 0.43 ± 0.04 , and 0.36 ± 0.03 mites to SS, EDL, and EP, respectively (Figure 1). No significant differences were found in percentage of groomed mites before and after the treatments (t statistic = 1.92, 0.86, and 0.34, and P = .15, .44, and .75 for SS, EDL, and EP, respectively). The percentage of groomed *Varroa* after the end of the treatment period were insignificantly higher to SS than the treatments with EP and EDL (DF = 2, F = 3.27, P = .08 > .05).

The percentage of dead bees increased from 0.00 ± 0.00 , 3.00 ± 0.01 , and $4.00 \pm 0.03\%$ at day 2 to 16.00 ± 0.02 , 29 ± 0.04 , and $54 \pm 0.07\%$ at day 7 for SS, EDL, and EP, respectively (Figure 2). The highest percentages of dead bees from day 2 to day 7 were to EP followed by EDL and finally SS. The percentages of dead bees from day 2 to 7 were significantly higher to EP than EDL and SS (DF = 2, F = 7.87, P = .0008 < .05). Similarly, at day 7 alone EP differed significantly than EDL and SS (DF = 2, F = 15.28, P = .0013 < .05).

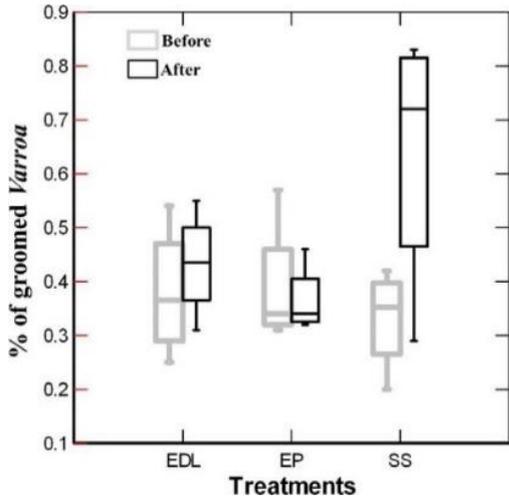


Figure 1: Variations in percentage of groomed *Varroa* after and before the treatment. SS: control group, EDL: drone larvae extract, EP: propolis extract. Median and interquartile are shown.

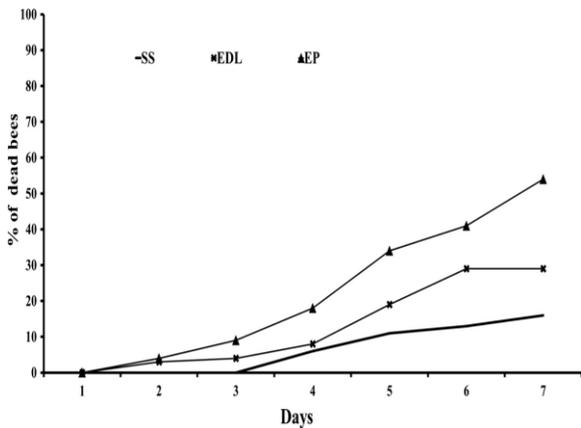


Figure 2: Mean percentage of dead bees for test groups over 7 days. SS: control group, EDL: drone larvae extract, EP: propolis extract.

The estimated survival means were 6.68 ± 0.11 , 6.33 ± 0.15 , and 5.90 ± 0.20 days for SS, EDL and EP, respectively. EP had the lowest survival than SS and EDL (Figure 3) and differed significantly less than SS (Mantel-Cox test = 18.89, $P = .000 < .05$) and EDL (Mantel-Cox test = 7.06, $P = .008 < .05$) while no significant differences were found between SS and EDL (Mantel-Cox test = 2.94, $P = .086 > .05$).

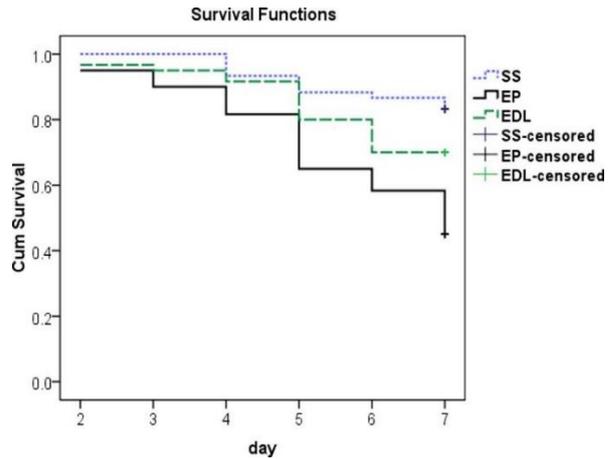


Figure 3: Cumulative survival over 7 days for test groups. SS: control group, EDL: drone larvae extract, EP: propolis extract.

Fluctuations in the Grooming Behavior

Number of counted *Varroa* mites dropped from 318 in November to 119 in February. The 2nd week of November had the highest number of counted *Varroa* (94 mites) while 2nd and 4th weeks of February had the lowest numbers (23 mites) as in Table 1. The weekly variations in the numbers of normal and groomed *Varroa* were not significant ($DF = 15, F = 0.88, P = .58$) and ($DF = 15, F = 1.17, P = .31$), in respect. Also, weekly variations in percentages of groomed *Varroa* were insignificant ($DF = 15, F = 0.93, P = .53$). The monthly variations were insignificant in case of normal *Varroa* mites ($DF = 3, F = 2.53, P = .06$) while in case of groomed *Varroa* and percentage of groomed *Varroa* were significant ($DF = 3, F = 3.59, P = .016$) and ($DF = 3, F = 3.15, P = .029$), respectively. February had significantly the least number of normal *Varroa* mites while December and February had significantly the least number of groomed *Varroa* mites. It is clear from the percentage of groomed *Varroa* mites that grooming behavior of the colonies was stable during the study period (i.e. no significant weekly variations were detected). Only December was significantly the least month in percentage of groomed *Varroa* based on the overall mean (Table 1).

Fluctuations in *Varroa* Morphology

The body length ranged from 1.03 mm at 1st week of January to 1.11 mm at 1st week of November with

difference of 0.08 mm. Body width ranged from 1.62 mm at 1st week of January to 1.69 mm at 1st week of November with difference of 0.07 mm. Body ratio ranged from 1.49 at 2nd week of December, 3rd and 4th weeks of February to 1.53 at 1st week of December, 2nd week of January and February with difference 0.04. It is clear that means of measured body characteristics declined significantly from 1st week of November to

January/February (Table 2). The variations in body length, body width and body ratio were significant on the weekly basis (DF = 15: F = 3.50, P ~ .0001; F = 3.66, P ~ .0001 and F = 2.11, P = .008, respectively) and were not significant on the monthly basis (DF = 3: F = 0.59, P = .62; F = 0.49, P = .69 and F = 0.72, P = .53, respectively) as in Table 2.

Table 1: Weekly and monthly variations (Means \pm SD) in numbers of normal and groomed *Varroa* mites and percentage of groomed mites. Means with the same letters are not significantly different according to Duncan Multiple Range test_{0.05}.

Month	Week	Total <i>Varroa</i> Number	Normal <i>Varroa</i>	Groomed <i>Varroa</i>	Percentage of groomed <i>Varroa</i>
November	1	66	3.71 \pm 4.34a	5.71 \pm 5.99a	51.12 \pm 29.79a
	2	94	9.71 \pm 9.65a	3.71 \pm 3.35ab	31.02 \pm 33.97a
	3	89	9.28 \pm 8.75a	3.42 \pm 3.20ab	36.53 \pm 33.11a
	4	69	6.71 \pm 10.01a	3.14 \pm 4.05ab	41.94 \pm 16.20a
Overall mean		318	7.35 \pm 8.37a	4.00 \pm 4.18a	39.20 \pm 28.61a
December	1	82	10.00 \pm 12.22a	1.71 \pm 1.79ab	17.03 \pm 13.53a
	2	79	9.71 \pm 12.90a	1.57 \pm 2.07ab	11.95 \pm 16.76a
	3	55	7.83 \pm 8.77a	1.33 \pm 1.50b	14.89 \pm 15.00a
	4	37	4.16 \pm 3.81a	2.00 \pm 2.75ab	25.00 \pm 21.51a
Overall mean		253	8.07 \pm 9.97a	1.65 \pm 1.95b	16.59 \pm 15.87b
January	1	33	3.66 \pm 6.65a	1.83 \pm 2.13ab	45.47 \pm 28.48a
	2	45	4.16 \pm 3.65a	3.33 \pm 4.50ab	34.32 \pm 41.33a
	3	63	6.50 \pm 5.61a	4.00 \pm 4.14ab	32.36 \pm 18.84a
	4	54	6.00 \pm 6.29a	3.00 \pm 3.63ab	29.46 \pm 18.58a
Overall mean		195	5.08 \pm 5.42ab	3.04 \pm 3.55ab	33.96 \pm 26.64ab
February	1	32	4.00 \pm 3.94a	1.33 \pm 1.96b	17.22 \pm 21.12a
	2	23	2.83 \pm 3.18a	1.00 \pm 1.26b	26.46 \pm 20.48a
	3	41	3.33 \pm 2.65a	3.50 \pm 3.39ab	41.97 \pm 24.64a
	4	23	1.66 \pm 1.03a	0.50 \pm 0.83b	16.66 \pm 23.56a
Overall mean		119	7.35 \pm 8.37b	4.00 \pm 4.18b	25.11 \pm 23.28ab

Table 2: Weekly and monthly variations (Means \pm SD) in body length, width and body ratio (body width/body length) of *Varroa* mites. Means with the same letters are not significantly different according to Duncan Multiple Range test_{0.05}.

Month	Week	Number	Body length (mm)	Body width (mm)	Body ratio
November	1	37	1.11 \pm 0.04a	1.69 \pm 0.06a	1.51 \pm 0.06bc
	2	40	1.09 \pm 0.03abc	1.67 \pm 0.04abc	1.52 \pm 0.06abc
	3	54	1.07 \pm 0.04bc	1.63 \pm 0.05de	1.51 \pm 0.07bc
	4	36	1.06 \pm 0.05c	1.64 \pm 0.06cde	1.54 \pm 0.08abc
Overall mean		167	1.08 \pm 0.04a	1.66 \pm 0.06a	1.52 \pm 0.07a
December	1	66	1.07 \pm 0.04bc	1.65 \pm 0.05bcde	1.53 \pm 0.07abc
	2	57	1.09 \pm 0.04abc	1.64 \pm 0.05cde	1.49 \pm 0.07c
	3	39	1.08 \pm 0.04bc	1.65 \pm 0.04bcde	1.52 \pm 0.06abc
	4	13	1.08 \pm 0.05bc	1.68 \pm 0.06ab	1.55 \pm 0.08ab
Overall mean		175	1.08 \pm 0.04a	1.65 \pm 0.05a	1.52 \pm 0.07a
January	1	11	1.03 \pm 0.06d	1.62 \pm 0.04e	1.57 \pm 0.09a
	2	25	1.09 \pm 0.02abc	1.67 \pm 0.05abc	1.53 \pm 0.05abc
	3	29	1.09 \pm 0.02abc	1.67 \pm 0.05abcd	1.52 \pm 0.05bc
	4	38	1.08 \pm 0.04bc	1.64 \pm 0.06cde	1.51 \pm 0.06bc
Overall mean		103	1.08 \pm 0.04a	1.65 \pm 0.06a	1.52 \pm 0.06a
February	1	19	1.09 \pm 0.02abc	1.66 \pm 0.04abcde	1.51 \pm 0.04bc
	2	19	1.07 \pm 0.04bc	1.66 \pm 0.04abcde	1.53 \pm 0.06abc
	3	23	1.09 \pm 0.02abc	1.63 \pm 0.05cde	1.49 \pm 0.04c
	4	10	1.10 \pm 0.04ab	1.65 \pm 0.07bcde	1.49 \pm 0.07c
Overall mean		71	1.09 \pm 0.03a	1.65 \pm 0.05a	1.51 \pm 0.05a

Discussion

Effects of Sugar Syrup (SS), Extracts of Drone Larvae (EDL) and Propolis (EP) on *Varroa* Mites and Honey Bees

The EP showed slight efficacy over SS or EDL in regard to increase the number of fallen mites. This can be attributed to the lethal effect of EP on *Varroa* mites especially that the narcotic and lethal effects of propolis on *Varroa* mites were found. At low concentrations of propolis (e.g. 5%) heat production rate of mites was passively impacted. Also, treatment with 5% propolis caused 100% inactive mites after 30 s. Using 4% propolis was able to impact metabolic activity of *Varroa* and cause death to the treated mites. Propolis was able to kill from 60.5% to 90% of mites after 30 s of exposure. The ability of all treatments including SS alone in

dropping mites can be explained by the active action of bees to clean their bodies from the syrup and hence dropping the mites.

The results showed that EP was more fatal to bees than EDL or SS over the 7 days. Under field conditions, it is expected that the bees will not be exposed continuously to large amounts of the treatment materials either EP or EDL as done in the laboratory experiment. Accordingly, 78% of mites were killed without any negative impacts on the bees when 10% propolis solution was used as spray over infested bees with *Varroa*. However, it is better to apply SS only under field conditions to avoid any potential adverse impacts on the bees. Moreover, EP and EDL did not greatly impact *Varroa* than SS. Also, intensive spraying is not recommended.

Fluctuations in the Grooming Behavior

The number of counted *Varroa* mites dropped from 318 in November to 119 in February. Accordingly, Narendra et al. (2016) found the lowest mean of mite population was in January and February. This can be attributed to the low activities of bee colonies in regard to brood rearing during the study period especially from December to February. The low brood rearing activity had a negative impact on *Varroa* reproduction and hence the number of detected mites towards the end of the study period. According to Narendra et al. (2016), a significant positive correlation of 0.853 and 0.887 was found between mean of *Varroa* population and maximum and mean temperature, respectively. Therefore, the low temperature towards the end of the study period (from autumn to winter) could impact the *Varroa* population passively than the beginning of the study. No significant weekly variations in percentages of groomed mites were detected, suggesting that grooming behavior of the colonies did not change during the study period. This reflects that the same group of bees (i.e. winter bees) had approximately the same grooming level during the winter time. The colonies had a little chance to renew their bee population due to the low brood rearing activity in the winter.

Fluctuations in *Varroa* Morphology

Body length, body width and body ratio of *Varroa* mites declined significantly from November until January/February on the weekly basis. This reflects that the morphology of *Varroa* is not stable in the course of time. The negative changes in the morphology of *Varroa* perhaps were due to the low feeding and activities of *Varroa* as a result of the low brood rearing activity of honey bees during winter. The variations in *Varroa* characteristics over time are supported by the study of Akimov et al. (2004). They found high similarity between morphology of winter and spring *Varroa* mites while summer samples differed significantly than the other seasons in some characteristics. The monthly variations for measured characteristics were not significant because the monthly means merged the weekly high and low values.

Conclusion

Spraying bees with sugar syrup can help controlling *Varroa* mites with less passive impacts on bees than propolis extract and extract of drone larvae. It is expected that spraying bees with sugar syrup on regular basis during colony inspection can help reducing the population of *Varroa* mites within colonies. Winter can be considered as a perfect time to assess the grooming behavior of honey bees due to the relative stability of this parameter during this specific season. Bee breeders can select the colonies with high grooming potential during winter to rear queen from them to obtain bee colonies with a natural ability to fight *Varroa*. It is better to assess the morphological characteristics of *Varroa* mites at different time points due to their fluctuation over time.

References

- Abdelghany, T.M., M.A. El-Naggar, M.A. Ganash and M.A. Al Abboud. 2017. PCR identification of *Aspergillus niger* with using natural additives for controlling and detection of malformins and maltoryzine production by HPLC. *Bionanoscience*, 7 (4): 588-596.
- Abdella, A., T.E. Mazeed, A.F. El-Baz and S. Yang. 2016. Production of beta-glucosidase from wheat bran and glycerol by *Aspergillus niger* in stirred tank and rotating fibrous bed bioreactors. *Process Biochemistry*, 51 (10): 1331-1337.
- Abdel-Naby, M.A., A.B. El-Tanash and A.D.A. Sherief. 2016. Structural characterization, catalytic, kinetic and thermodynamic properties of *Aspergillus oryzae* tannase. *International Journal of Biological Macromolecules*, 92: 803-811.
- Ahmad, F.B., Z. Zhang, W.O.S. Doherty and I.M. O'Hara. 2016. Evaluation of oil production from oil palm empty fruit bunch by oleaginous microorganisms. *Biofuels Bioproducts & Biorefining-Biofpr*, 10 (4): 378-392.
- Ahmad, F.B., Z. Zhang, W.O.S. Doherty and I.M. O'Hara. 2017. Microbial oil production from sugarcane bagasse hydrolysates by oleaginous yeast and filamentous fungi. *International Sugar Journal*,

- 119 (1417): 30-35.
- Ajaib, M., T. Arooj, K.M. Khan, S. Farid, M. Ishtiaq, S. Perveen and S. Shah. 2016. Phytochemical, Antimicrobial and Antioxidant Screening of Fruits, Bark and leaves of Lagerstroemia indica. *Journal of the Chemical Society of Pakistan*, 38 (3): 538-545.
- Akimov, I.A., Benedyk, S.V., Zaloznaya, L.M., 2004. Complex analysis of morphological characters of gamasid mite Varroa destructor (Parasitiformes, Varroidae). *Vest. Zool.* 38, 57–66.
- Aleku, G.A., S.P. France, H. Man, J. Mangas-Sanchez, S.L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N.J. Turner. 2017. A reductive aminase from *Aspergillus oryzae*. *Nature Chemistry*, 9 (10): 961-969.
- Ali, I., M. Zafar, M.I. Anwar, M. Irshad, Z. Anwar, A. Ahmad and H. Nawaz. 2017. KINETIC CHARACTERIZATION and INDUSTRIAL APPLICABILITY of NOVEL PROTEASE PRODUCED FROM aspergillus ornatus USING AGRO-INDUSTRIAL MATERIALS. *Cellulose Chemistry and Technology*, 51 (1-2): 137-144.
- Ali, S. and W. Nawaz. 2016. Biotransformation of l-tyrosine to dopamine by a calcium alginate immobilized mutant strain of aspergillus oryzae. *Applied Biochemistry and Biotechnology*, 179 (8): 1435-1444.
- Ali, S. and W. Nawaz. 2017. Optimisation of nutritional requirements for dopamine synthesis by calcium alginate-entrapped mutant strain of *Aspergillus oryzae* EMS-6. *Natural Product Research*, 31 (3): 281-288.
- Amin, H.I.M., A.A. Amin, S. Tosi, G. Giacomo, F.H.S. Hussain, A.M. Picco and G. Vidari. 2017. Chemical composition and antifungal activity of essential oils from flowers, leaves, rhizomes, and bulbs of the wild iraqi kurdish plant iris persica. *Natural Product Communications*, 12 (3): 441-444.
- Aumeier, P., 2001. Bioassay for grooming effectiveness towards Varroa destructor mites in Africanized and Carniolan honey bees. *Apidologie* 32, 81–90.
- Farooqi, Z.U.R., M.S. Nasir, A. Nasir, N. Zeeshan, I. Ayub, H. Rashid, M.U. Qamar, A. Sarwar and M.A. Akram. 2017. Evaluation and analysis of traffic noise in different zones of Faisalabad – an industrial city of Pakistan. *Geology, Ecology, and Landscapes*, 1 (4): 232-240.
- Narendra, G., Chaudhary, O.P., Kaushik, H.D., 2016. Evaluation of ectoparasitic brood mite, Varroa destructor detection techniques and its seasonal population dynamics. *Ann. Plant Prot. Sci.* 24, 254–258.