

BEE POLLEN AND PROPOLIS:

The Hidden Treasure to Fight Back Covid-19



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The Hidden Treasure To Fight Back Covid-19

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DEDICATED TO
ALL THE PEOPLE WHO HAVE LOST
THEIR LIFE DUE TO COVID-19
AND WHO ARE FIGHTING WITH
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3 May 2021



FOREWORD

COVID-19 is emerged as the biggest crisis of 21st century and it still stands as challenge for existence of civilization. Society, scientists, spiritual world and government all are fighting with epidemic in their own ways. At this difficult time any small contribution towards fight against pandemic worth to be recognized as is contribution to the civilization. According to World Health Organization (WHO) SARS-CoV-2 virus has already infected more than 152 million people and accounted for death toll of around 3.19 million till date and there is no sign of abating in near future. Still no proven treatment protocol till date.

This is time to tailor the indigenous knowledge to manage and minimize the damage from dreaded pandemic. In this endeavor, the efforts made by Dr. H.S. Baweja by bringing out a timely publication titled "**Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19**" is worth appreciation.

The book provides knowledge on various aspects related to medicinal properties of pollen and propolis. Scientific evidences have been quoted that Bee Pollen which is the bee product with high amounts of quercetin, kaempferol and its derivates can be a promising alternative to immunity building to fight against COVID-19 and post Corona care. I am also made aware that propolis consumption can also reduce the risk of diabetes, cardiovascular problems, kidney diseases and has antibacterial/antiviral properties and thus can help in treatment of COVID-19 patients. Since pollens are fine powder like substances produced by flowering plants and collected by bees and are reported to be composed of proteins, essential amino acids, reducing sugars, lipids, nucleic acids, crude fiber, minerals, vitamins, vitamin E, niacin, thiamine, biotin, folic acid, enzymes, phytosterols, flavonoids and organic carotenoid pigments etc. and are capable to strengthen our immunity, which is the need of the hour to fight dreaded Corona Virus.

Bee keeping has potential to become not only good source of farm income but also possess vast floral diversity. The Government's schemes are supporting the bee keeping activities in the big way, the only need is to translate the efforts into enhanced area and number of units as farm level entrepreneurs. This will results into increase in honey production and flower production directly and increase in over-all fruit and vegetable production through role of Honey bee as pollinators. Finally the work is also in consonance with the vision of Hon'ble Prime Minister of India as it contributes to increase the Farm Income. I do hope that the Directorate of Horticulture and Food Processing, Uttarakhand will continue to work further in this direction.

Lastly, I extended my best wishes to Dr. Baweja for very timely publication in this hour of need.

(Dr. Harbans Singh Chugh)



PREFACE

Pollen is a fine powder produced by flowering plants and collected by bees. When mixed with nectar (and/or honey) and salivary substances it is used as the main food reserve for the hive. Bee pollen is a raw material from which bees produce bee bread. They collect pollen from plant anthers, mix it with a small dose of the secretion from salivary glands or nectar and place it in specific baskets (corbiculae) which are situated on the tibia of their hind legs. These are called pollen loads. In the hive the collected pollen dampened with saliva and fragmented by flightless bees is packed in honeycomb cells. Next the surface of the collected pollen is covered with a thin layer of honey and wax. The substance which has been created is bee bread which undergoes anaerobic fermentation. Bee pollen is an energy food used by humans as a diet supplement and for the conditioning of athletes. The high content of protein, fat and minerals gives bee pollen a nutritional value similar to or higher than that of dried legumes. A wide range of therapeutic properties have been suggested including antimicrobial, antiviral, antioxidant, hepatoprotective, chemopreventive, anticarcinogenic, antiallergenic, antiatherosclerotic, anti-inflammatory and immunomodulatory activities. The book on "Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19" has eight chapters. The first chapter, introduction includes honey bee and human relationship, pollen in bee flower relations and nectar. The second chapter, structure and composition includes pollen structure, primary metabolites, chemical composition of pollen, carbohydrates and reducing sugars, fatty acids, mineral matter, lipids, proteins, amino acids, vitamins, nucleic acids, water, enzymes and secondary metabolites. The third chapter, health benefits includes health benefits of bee pollen, antioxidant activity, antimicrobial activity, anti-inflammatory activity, anticancer activity, anti-atherosclerotic and anti-diabetic properties, antiallergic activity, nutritional values and health hazards. The fourth chapter, bee pollen and propolis: A remedy for Covid-19 includes bee pollen and propolis as a remedy for COVID-19 (SARS-CoV-2), bee pollen, propolis, propolis extraction from hives, commercial traps, non-commercial propolis traps, hive scrapings, chemical composition of propolis, characteristics and bioactive composition of propolis, characteristics, composition, comorbidities in COVID-19 patients as well as evidence that propolis can help to mitigate their impact, cancer, cardiovascular and hypertension disease, obesity, old age, diabetes, kidney diseases, bacterial infection, mechanism, effect on SARS-CoV-2, doses and *Cannabis sativa* pollen. The fifth chapter, pollen source includes monofloral and polyfloral bee pollen. The sixth chapter, benefits of pollen for honey bee health includes diet requirements according to the age of the honey bee and importance of pollen, impact of pollen compounds on bee health and resistance to pesticides and diseases, pollen quantity, pollen quality, the influence of pollen quality on nosema tolerance, pesticides resistance and beekeepers feed management. The seventh chapter, pollen collection and beehive includes pollen trap, pollen collection, botanical origin of pollen and langstroth beehive. The eight chapter, processing includes bee pollen processing, routes of administration and dosing, bee bread, the



application of pollen as a functional food ingredient, the effect of drying techniques and storage conditions on the quality of bee collected pollen, the influence of drying techniques on the quality of bee collected pollen, fermented bee collected and floral pollen based products, spring festival 2021 (Uttarakhand) and source of employment. The book was written keeping in view the needs of farmers and people in mind who are battling COVID-19 disease with high spirit.

Padam Shri Dr. Mahesh Sharma

Chairman, Shivalik Mission

Chancellor, Mahatma Gandhi Central University, Motihari, Champaran

Ex Chairman, KVIC

Er. D. P. Singh, IIT Roorkee

The Transformer of Indian Honey Scenario

Shri Vijay Kumar Goel

Chairman, Dhampur Sugar Mills Ltd.



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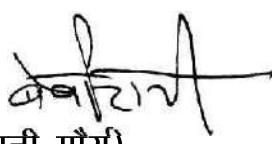


संदेश

मुझे यह जानकर प्रसन्नता हुई कि उद्यान एवं खाद्य प्रसंस्करण विभाग, उत्तराखण्ड द्वारा “Bee Pollen and Propolis : The Hidden Treasure to Fight Back Covid-19” विषय पर प्रकाशन किया जा रहा है।

शहद एक संतुलित खाद्य है तथा इसमें विभिन्न औषधीय गुण हैं। शहद प्रतिरक्षा क्षमता को सुदृढ़ करता है। मधुमक्खी पराग भी स्वास्थ्य के लिए लाभकारी माना जा रहा है। कोविड-19 जैसी महामारी में प्रतिरक्षा प्रणाली को मजबूत रखने हेतु शहद तथा अन्य मधुमक्खी उत्पादों का महत्व बढ़ गया है। प्रधानमंत्री जी द्वारा भी मधुमक्खी पालन को प्रोत्साहित किया जा रहा है। शहद उत्पादन के माध्यम से किसानों की आय बढ़ेगी तथा “आत्मनिर्भर भारत अभियान” को भी मजबूती मिलेगी। राज्य में किसानों को मौनपालन हेतु प्रेरित करना आवश्यक है। शहद प्रसंस्करण हेतु सुविधायें भी उपलब्ध करवानी होंगी। उत्तराखण्ड के जैविक शहद को राष्ट्रीय एवं अन्तर्राष्ट्रीय बाजारों से जोड़ना भी आवश्यक है। आशा है कि उद्यान एवं खाद्य प्रसंस्करण विभाग, उत्तराखण्ड द्वारा उक्त विषय पर प्रकाशन से किसानों में मधुमक्खी पालन के प्रति जागरूकता बढ़ेगी।

सफल प्रकाशन हेतु हार्दिक शुभकामनायें।



(बेबी रानी मौर्य)



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MESSAGE

COVID-19 Pandemic has reached to more than 150 nations and has caused WHO to call the disease a worldwide problem. Humanity is confronting it as there is no proven treatment. At the same time the information provided by the Department of Horticulture and Food Processing, Govt. of Uttarakhand through a timely publication "**Bee Pollen and Propolis: The Hidden Treasure to Fight Back COVID-19**" quoting therein that Bee Pollens are capable to fight against the pandemic, may prove to be a sigh of relief to the humanity. Though it is for our scientists to go deep into it and validate the effectiveness of this knowledge to the large mass.

Moreover, the knowledge of honey production through beekeeping is known to us for centuries, both for food supplement and its medicinal values, as plenty of references are available in our vedic literature. In India, it is commonly referred as **MADHU** and so in ayurvedic scriptures for its medicinal values. Having realizing its importance, now this is the high time to enlarge its production not only for its medicinal values but also to maintain our ecosystem, to improve fruit production as bees are the best nature's pollinators and also to supplement the income of our farmers/beekeepers. This is the mission and dream of Hon'ble Prime Minister of India.

The Government of Uttarakhand will be happy to support the objectives of National Beekeeping & Honey Mission for the overall promotion & development of scientific beekeeping in the state to achieve the goal of 'Sweet Revolution' & Bee Flora development at all level.

I do hope that Directorate of Horticulture & Food Processing would formulate a suitable project programme on bee keeping and implement it in "letter and spirit" to increase the income of farmers.

With good wishes,

(Tirath Singh Rawat)



सुबोध उनियाल

मंत्री

Subodh Uniyal

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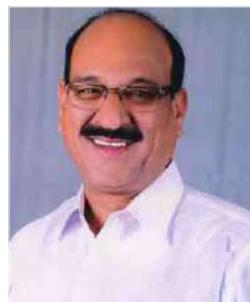
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MESSAGE

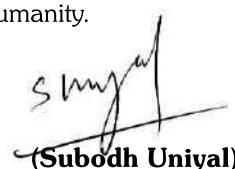
We are aware that the Covid-19 has led to a dramatic loss of human life across the world and presents an unprecedented challenge to public health, food systems and the world of work. The economic and social disruption caused by the pandemic is devastating. As on date, there is no exact treatment for this virus and therefore scientists are toiling hard to come up with solutions, though they may be in the process of reaching to a conclusive stage but it may take some more time as various protocols will have to be observed prior treatment comes to the public.

It gives me pleasure to note that Directorate of Horticulture & Food Processing, Govt. of Uttarakhand has brought a publication on “Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19”, where it has been documented that bee pollens can fight well against dreaded Corona Virus. This is a matter of relief to us as well as food for thought for our scientist fraternity to validate its penetration against Covid-19. Nevertheless use of pollens or honey doesn't cause any harm to human health as long they are used judiciously. I am also aware about the medicinal properties of honey as it is capable in treating various ailments like treatment of eye diseases, cough, thirst, phlegm, hiccups, blood in vomit, leprosy, diabetes, obesity, worm infestation, vomiting, asthma, diarrhoea and healing wounds etc.

As regards Uttarakhand, it has rich wide biodiversity where at one side nature is performing its cycles like honey production is going on in the forests, which is collected and used for various purposes including medicines and at another side there is tradition of honey production in our hills as people used to make a provision of locally known as **Khadra (wall hives)** in their houses to provide shelter to honey bees to continue their life cycle and in return they harvest honey for their domestic consumption.

Since Uttarakhand has wide diversity of Flora & Fauna, tradition of honey production, ready market, Govt. policies akin to large scale honey production and therefore there is need to make honey production a mass drive to generate volume, improve income of producers and provide immunity booster to the people against Corona virus.

I do hope that Directorate of Horticulture & Food Processing, Govt. of Uttarakhand will encash this golden opportunity not only to improve the income of farmers but also to serve the humanity.



(Subodh Uniyal)

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Justice Raghvendra S. Chauhan
CHIEF JUSTICE



HIGH COURT OF UTTARAKHAND
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MESSAGE

In Rajasthan, there is an old saying that “*when one loses a camel, the owner would look for the camel even in a pot*”.

Faced with the monstrosity of pandemic, which is sweeping through the Nation and this State, every endeavor is being made to defeat the pandemic and to rescue our people. Unknown to most of us, nature may hold the cure for the dreaded virus. Therefore, the present book, “*Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19*”, emerges as a practical treatise for fighting Covid-19 pandemic.

The book is an exhaustive one: while it deals with the history of the inter-relationship between the honey bee and the human beings, it also deals with the biological aspects, such as structure and composition of a pollen. The book also contains details about pollen collection and processing. In order to illustrate the various topics, the book is supported by excellent figures, illustrations and tables.

The book is certainly a pioneering work, which would benefit not only the scientific community, but would also teach the farmers how to diversify their agricultural activities and to include “Apiculture” as part of their agricultural work. Moreover, since India has the benefit of diverse flowers, which bloom throughout the year, in different seasons, Bee Pollen collection and processing needs to be encouraged and marketed. This sector of agriculture holds an immense potential for placing India on the agricultural world map. If Horticulture and Apiculture were to be encouraged by the Government, it would create large number of self-employment for our people.

I would certainly like to congratulate the learned authors for their in-depth research and presentation of the subject in clear and concise manner. I would also recommend that the book should be translated into other regional languages so that the knowledge can be shared equally with other regions of the country.


(Justice R.S. Chauhan)

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ओम प्रकाश
Om Prakash



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MESSAGE

It is heartening to note that Department of Horticulture and Food Processing, Uttarakhand is going to publish a book on "**Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19**". The book contains rich information on various aspects involved in honey production with scientific explanations. I am aware about the medicinal uses of honey in treating various diseases and ailments but **eye opening information is that Bee Pollens are capable to fight against Corona virus.**

At this hour of need, this information can provide an agenda to our scientists/researchers that are working day and night in search of a solution to combat Corona Virus.

Moreover under agriculture diversification policy of the Government honey production is one of the thrust areas. Since we have learnt that bee pollens are capable to fight against dreaded Corona Virus so there is need to popularize use of bee-pollen/honey and make it one of the ingredients of our daily food not only to combat Corona Virus but also to improve immunity. Based on prevailing experience, this is the need of the hour. Though this task is being executed very well by large production/business houses of honey through electronic/print media but still there is shortfall. So to cater global market demand there is need to gear up honey production.

In this endeavour Directorate of Horticulture and Food Processing can capitalize this opportunity by formulating a work plan to increase production of honey and its demand driven ingredients. Bee keeping also has a merit that it is seasonal in nature and does not have too long gestation period. There is also a need for world class processing facilities so that our products could reach to various global destinations without any if and buts. Thrust is required to be given on organic production and certification for value addition and purity.

So Directorate of Horticulture & Food Processing may set its agenda accordingly.

I do hope that this timely publication will be helpful in setting up of the state road map for honey production which will not only improve the income of producers but also will help in achieving the Government Policy objective.

I wish the publication a success.

3.5.21
(Om Prakash)



Dr. Harminder Singh Baweja

Director, Department of Horticulture and Food Processing, Govt. of Uttarakhand
Chief Executive Officer, Uttarakhand Horticulture Board



ACKNOWLEDGEMENT

Words fall short of expressing one's emotions and feelings, mainly when one sits in acknowledging the debts of others. Firstly, I would like to praise and thank the 'Almighty' - the supreme power for helping me in all adversities, at every step, at each moment.

I am overwhelmed with joy to evince my profound sense of reverence and gratitude to Smt Baby Rani Maurya, Honourable Governor of Uttarakhand, for her inspiring support, guidance and continuous encouragement through the preparation of the book. I express a deep sense of gratitude to Shri Tirath Singh Rawat, Honourable Chief Minister, Shri Subodh Uniyal, Agriculture and Farmer Welfare Minister, Government of Uttarakhand for their guidance and support, not only in writing and publishing this book but also to the Department of Horticulture and Food Processing, Uttarakhand. They are striving hard to develop Uttarakhand into a Horticultural Hub of Himalayan States.

I would like to express my sincere gratitude to the Honourable Chief Justice of Uttarakhand High Court, Shri R. S. Chauhan, for his time to time guidance and motivation helped me accomplish the task without wasting much time.

It would not have been possible to publish this book without the guidance, encouragement and support of Shri Om Prakash, Chief Secretary, Smt Manisha Panwar, Additional Chief-Secretary and Dr Harbans Singh Chugh, Secretary, Agriculture and Farmer Welfare, Government of Uttarakhand.

I feel indebted to Dr Radha Krishana Pant, Secretary, FORD, Major Mudit Sood, ADC to Honourable Governor of Uttarakhand, Shri Awadesh Verma, IIT Roorkee, Shri Vineet Narain, Braj Foundation, Shri Arun Tyagi, Action for Development Foundation, Shri Yogender Pal Singh, President, Indian Nurserymen Association, Prof. Arvind Shukla, GBPUA&T, Pant Nagar, Shri Kolipaka Srinivas, Shri Ajay Saini, Shri Yoginder Punia for providing amenities during my entire course of investigations.

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Words in lexicon would be a few exiguous to express my deep sense of gratitude for my loving mother, Smt Gurbans Kaur, for her selfless sacrifices and heartfelt blessings throughout my life. I owe an enormous debt of gratitude to my beloved wife, Dr Parminder Kaur Baweja, who gave me detailed and constructive comments on one or more chapters. I also admire the support provided by my sons Prabhsimran and Anukirat. They gave generously of their time to interpret the text from the user's point of view and pushed me to simplify ideas, discuss particular aspects of insight, and justify the rationales for various suggestions.

I wish to thank the entire Baweja Family, all well-wishers, whose blessings propelled me to achieve my dreams and could not find a separate mention due to lack of space.

(Dr. Harminder Singh Baweja)



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Table 3	:	Details of Bee Flora a Source of Pollen and Nectar (As per Indian Conditions)



ABOUT THE BOOK

The book on Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19 has eight chapters. The first chapter, introduction includes honey bee and human relationship, pollen in bee flower relations and nectar. The second chapter, structure and composition includes pollen structure, primary metabolites, chemical composition of pollen, carbohydrates and reducing sugars, fatty acids, mineral matter, lipids, proteins, amino acids, vitamins, nucleic acids, water, enzymes and secondary metabolites. The third chapter, health benefits includes health benefits of bee pollen, antioxidant activity, antimicrobial activity, anti-inflammatory activity, anticancer activity, anti-atherosclerotic and anti-diabetic properties, antiallergic activity, nutritional values and health hazards. The fourth chapter, bee pollen and propolis: A remedy for Covid-19 includes bee pollen and propolis as a remedy for COVID-19 (SARS-CoV-2), bee pollen, propolis, propolis extraction from hives, commercial traps, non-commercial propolis traps, hive scrapings, chemical composition of propolis, characteristics and bioactive composition of propolis, characteristics, composition, comorbidities in COVID-19 patients as well as evidence that propolis can help to mitigate their impact, cancer, cardiovascular and hypertension disease, obesity, old age, diabetes, kidney diseases, bacterial infection, mechanism, effect on SARS-CoV-2-doses and *Cannabis sativa* pollen. The fifth chapter, pollen source includes monofloral and polyfloral bee pollen. The sixth chapter, benefits of pollen for honey

bee health includes diet requirements according to the age of the honey bee and importance of pollen, impact of pollen compounds on bee health and resistance to pesticides and diseases, pollen quantity, pollen quality, the influence of pollen quality on nosema tolerance, pesticides resistance and beekeepers feed management. The seventh chapter, pollen collection and beehive includes pollen trap, pollen collection, botanical origin of pollen and langstroth beehive. The eighth chapter, processing includes bee pollen processing, routes of administration and dosing, bee bread, the application of pollen as a functional food ingredient, the effect of drying techniques and storage conditions on the quality of bee collected pollen, the influence of drying techniques on the quality of bee collected pollen, fermented bee collected and floral pollen based products, spring festival 2021 (Uttarakhand) and source of employment. Glossary containing related terms have been given along with selected references to obtain the information on Bee Pollen and Propolis: The Hidden Treasure to fight back Covid-19. The efforts have been made to describe the various chapters of Bee Pollen and Propolis: The Hidden Treasure to Fight Back Covid-19 in a systematic and comprehensive manner. The subject matter was illustrated with tables and figures wherever felt necessary. The book has been written keeping in view the needs of farmers and people who are fighting with high spirit against COVID-19 disease.



Chapter-1

INTRODUCTION

Honey bees are one of the most important and valued arthropods in world which served as breakthrough in challenging Darwin's theory of *Natural Selection* (Herb, 2014). The honey bees are ecologically and economically significant and provide pollination to around 80 per cent of major food crops (Decourtey *et al.*, 2019). Honey bees belong to family Apidae and Meliponidae of order Hymenoptera, and show characteristic eusocial behaviour exhibiting division of labour among its different castes (Winston, 1987) which is also referred as 'collective personality' (Wray *et al.*, 2011). In India some indigenous species of *Apis* are present *viz.*, *Apis cerana indica* Fabricius (The India bee), *Apis florea* Fabricius (The Little bee), *Apis dorsata* Fabricius (The Rock bee), *Apis andreniformis* Smith (The Black dwarf bee), *Apis laboriosa* Smith (The Himalayan Bee) and *Apis mellifera* Linnaeus (The Italian bee) is introduced one. *Tetragonula iridipennis* Smith which is also known as Dammer bee or stingless bee belong to family Meliponidae and is one of the emerging medicinal important bees. Due to the easy domestication and good honey gathering qualities *Apis mellifera* is one of the favourite honey bee of farmers all over the world.

Honey Bee and Human Relationship:

Honey bees are insects of great importance for humanity. Humans have taken advantage of them from ancient times both directly through bee keeping and indirectly through farming so their importance goes beyond ecology being also economic. Honey bees not only produce honey but also other products of great importance to humans. These products are beeswax, pollen, propolis and royal jelly. Worker bees secrete bee wax to form the combs and to seal cells when honey is stored. They can also store the pollen they collect from plants in order to feed brood. Besides, workers produce royal jelly which is a substance they use to feed newborn larvae and especially the queen. Propolis is made of different substances including resin and it is used to keep the hive free of pathogens. Men have taken advantage of honey bees from Antiquity. Honey was then the sweetener par excellence as sugar was unknown. Prehistoric men collected honey from natural honey bee hives. Honey bees are globally the most prominent pollinator used for pollination services in crops. The current widespread decline of insect pollinators (Potts *et al.*, 2010) could negatively affect human well being and food production as many crops rely on insect pollination for the quantity and quality of their yield (Potts *et al.*, 2016). To meet the demand for pollination services in crops it is common practice to introduce managed pollinators in particular colonies of the western honey bees (Garibaldi *et al.*, 2017). As humans we must recognise that the survival of bees is directly related to the survival of our habitats. Bees not only pollinate the flowers that we admire but they also fertilise the crops that we depend on for food. Because of this both bees and flowers have evolved and become more productive over their life cycles benefiting the human population.



Pollen in Bee Flower Relations:

For both the flower and the bee pollen is essential for life reproduction. Pollen is an important part of the bee diet and nectar is created when water is mixed with the sugar in the flower. Nectar is therefore essential for the bee survival. Nectar is a food source that gives bees the energy they need to travel from one plant to another for cross pollination. Flowers depend on bees to pollinate them. Pollen serves as the flowers seed which is essential for the species survival.

The colour, odour, shape, size, timing and reward (nectar or pollen) of a flower can influence the number of specific pollinators that visit it. Pollen and nectar are commonly clump together as floral rewards for pollinating bees but they serve entirely different functions for flowers and bees, as well as in their relationship. While flowers are specific for particular pollinators *via* nectar bees specialise on specific flowers through pollen. While pollen is required for pollination in flowers. It is an important larval food for bees. As a result, they are in fierce competition for pollen. Pollen foraging must be divided into three stages: uptake in the flower, reloading into and homeward transport within a carrying container. Bees have transport specialisations but few of them for pollen uptake and thus pollination. Bees that are actively harvesting pollen do not usually pollinate. This occurs only as a result of pollen contamination of the bee. Specialized bee flowers are frequently distinguished by their ability to hide pollen from bees while also utilising them as optimal pollinators. If there is any positive mutualism in the relationship between bees and flowers it is better defined as healthy altruism exploitation (Westerkamp, 1996).

Nectar:

Nectar is a sugar rich liquid produced by plants in glands called nectaries or nectarines either within the flowers with which it attracts pollinating animals or by extra floral nectaries which provide a nutrient source to animal mutualists which in turn provide herbivore protection. Common nectar consuming pollinators include mosquitoes, hoverflies, wasps, bees, butterflies, moths, humming birds, honey eaters and bats. Nectar is an economically important substance as it is the sugar source for honey. It is also useful in agriculture and horticulture because the adult stages of some predatory insects feed on nectar. Nectar secretion increases as the flower is visited by pollinators. After pollination the nectar is frequently reabsorbed into the plant.

Pollen is a fine powder that flowering plants produce and bees collect. When combined with nectar and salivary substances it serves as the primary food reserve for the hive (Denisow and Denisow-Pietrzylk, 2016; Thakur and Nanda, 2020). Bee pollen is a commodity used to produce bee bread by bees. Pollens are collected from plant anthers mixed with a small quantity of salivary or nectar gland and placed in special baskets (corbiculae) positioned their hind-legged tibia referred to as pollen loads. Pollens are gathered and transported to a hive through a field of bees (Couto and Couto, 2006, Pereira *et al.*, 2006). In the cells of honeycomb the hive is packaged pollen which is dampened with saliva and split by flightless bees. The pollen surface is then covered by a thin layer of wax and honey. The material is bee bread which is anaerobic and preserved by the produced lactic acid. The bee colonies primary source of protein is bee bread. Besides that, it is a source of mineral and nutritional substances for worker bees which produced royal jelly (Couto and Couto, 2006, Pereira *et al.*, 2006).

The world is suffering from COVID-19 pandemic which is acute respiratory syndrome with more than 81 million reported cases and around 1.8 million death by the end of 2020 (WHO, 2020). At this time of public health crisis Apitherapy is evolving as a promising source of COVID-19 treatment (Lima *et al.*, 2021). Apitherapy involves use of honey bee products such as honey, pollen, propolis, royal jelly, beeswax, and bee venom that showed potent antiviral activity against pathogens that cause severe respiratory syndromes (Hashem, 2020).



Chapter-2

STRUCTURE & COMPOSITION

Pollen Structure:

The structure of a pollen grain is very simple. The smallest pollen grain is obtained from the *Myosotis* which is 7 μm long and the largest pollen grain is found in the pumpkin *i.e.* 150 μm long (Figure 1). Pollen grain is made up of two cellular walls: an external wall called exine which is made up of sporopollenin and an internal wall called intine which is made up of polysaccharides and glycoprotein. The pollen grain consists of two cells *i.e.* a vegetative cell (responsible for pollinic tube development during the germination phase), a reproductive cell (which is involved in fertilization). Exine the external wall's solidity is resistant to putrefaction and digestion. As a result, nutrient compounds are difficult to

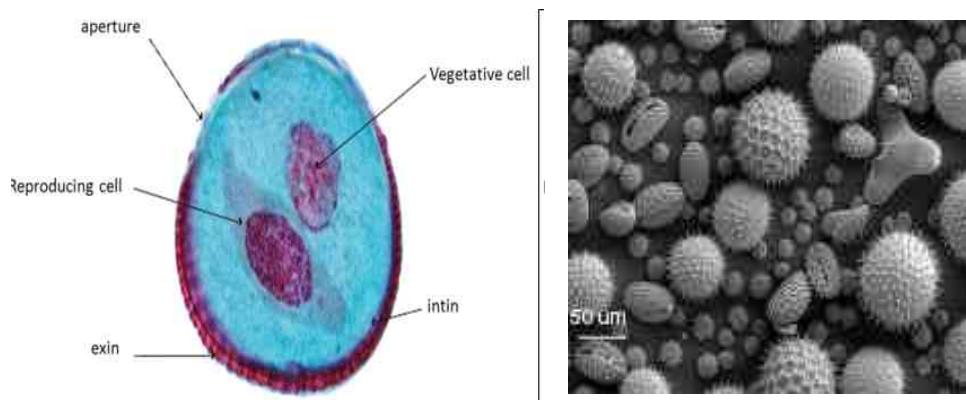


Figure 1: Pollen Structure and Pollen Diversity

access. Six strategies have been developed by animals to extract pollen compounds. Honey bees are said to be breaking this wall in spite of an osmotic shock while pollen is transported from the stomach into the ventricle, according to researchers (Roulston and Cane, 2000). Since both organs are subject to varying osmotic stress on both sides of the cell wall there are two distinct pressures that the cell cannot handle. The cell wall is therefore shattered mechanically (Kroon *et al.*, 1974). The exine is characterised by a high level of resistance to physicochemical influences. There are many pores and furrows on its top and also a coating of balsam which helps pollen adhere to bees abdomen (Couto and



Couto, 2006). Pollen grains vary in colour, shape, weight and size depending on the plant species. Grain shapes range from cylindrical to round, bell-shaped, triangular (Shubharani *et al.*, 2013). Their mass is equivalent to dozens of micrograms. Pollens consist of single grains, sometimes combined with two or more grains (Shubharani *et al.*, 2013). The pollen colours are ranging from bright yellow to black. The pollen basket brought to the hive is typically made up of pollen from a single plant. However, it is not strange for bees to collect pollen from a wide variety of plant species. Poppy, corn and lupine are examples of plants from which particular pollen is collected, whereas bees collect both nectar and pollen from other melliferous plants. Bees avoid collection of pollen from grass. They may however, collect fungal spores from mouldy plants on occasion (Wojcicki, 1987; Dubtsova, 2009).

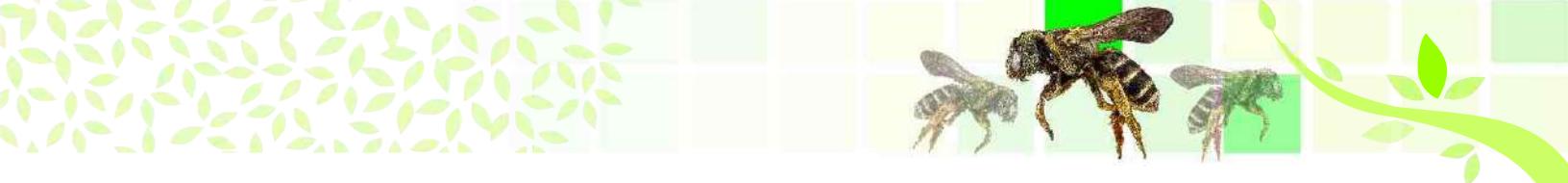
Primary Metabolites:

Primary metabolites are those compounds which are required for life to exist and to ensure growth, development and reproduction. Examples of primary metabolites include carbohydrates, lipids, minerals, proteins, vitamins and water. Secondary metabolites on the other hand are not as important as primary metabolites because they are naturally produced by plants. They do not play a direct role in normal growth, development or reproduction. Secondary metabolites like antibiotics have a long-term effect on reproductivity and survivability. They can act as transport agents (sexual hormones) as well as tissue reinforcement agents (cellulose and lignin).

Chemical Composition of Pollen:

Bee pollen consists of proteins (5-60 per cent), essential amino acids, reducing sugars (13-55 per cent), lipids (4-7 per cent), nucleic acids in particular RNAs, crudely fibre based acids (0.3-20 per cent) and minor substances such as minerals (Ca, Mg, Fe, Zn, Cu, K and Na), vitamins (provitamins (β -carotenes), vitamin E, niacin, thiamine, biotin and also follic acidification (Thakur and Nanda, 2020). The chemical composition of the bee pollen and many other factors like climatic conditions, soil type, the race and activities of the bees and other epitherapeutics which are greatly influenced by its own plant source and geographic origins (Nogueira *et al.*, 2012). About 250 substances including amino acids, lipids (triglycerides & phospholipids), vitamins, macro and micronutrients and flavonoids occur in bee pollen (Silva *et al.*, 2014; Nogueira *et al.*, 2012). Pollen is a natural plant product with high bioactive concentration. A total of 200 different substances comprised pollen grains from various plant species. The categories of essential chemical substances are proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzyme, coenzyme, vitamins and bioelements (Campos *et al.*, 2008; Campos *et al.*, 2010). On an average pollen contains 22.7 per cent of proteins which include, methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine and tryptophan 10.4 per cent of essential amino acids. These protein elements are vital to life and cannot be produced alone by the organism.

Furthermore, pollen contains significant amounts of nucleic acids particularly ribonucleic acids. On average 30.8 per cent of the pollen contains digestible carbohydrates. This product contains approximately 25.7 per cent reducing sugars primarily fructose and glucose (Kdzia and Hoderna-Kedzia, 2005). The essential fatty acids should be mentioned first among the lipids that are present in pollen in the amount of about 5.1 per cent. Phospholipids account for 1.5 per cent of the total while phytosterols particularly p-sitosterol account for 1.1 per cent (Szczesna, 2006). Another group included phenolic compounds which account for around 1.6 per cent of the total. Flavonoids, leukotrienes, catechins and phenolic acids are all members of this group. Among the flavonoids found in pollen 1.4 per cent are kaempferol, quercetin and isorhamnetin while 0.2 per cent are phenolic acids primarily chlorogenic acid (Asafova, 2001). Pollen is distinguished by a high concentration of triterpene bonds. Oleanolic acids, 3-ursolic acid and



betulin alcohol are the most common compounds (Kedzia and Hoderna-Kedzia, 2005; Kedzia and Hoderna-Kedzia, 2012). Pollens are good source of vitamins, fat-soluble 0.1 per cent like provitamin A, E and D while water-soluble 0.6 per cent like B1, B2, B6, C and acids: pantothenic, nicotinic, folic, biotin, rutin and inositol. Their gross contribution to the final product is 0.7 per cent. Bio elements account for approximately 1.6 per cent of the total and include macro nutrients (Ca, P, Mg, Na, and potassium) as well as micronutrients (Fe, Cu, Zn, Mn, Si and Se). The latter one present in the amount of 0.02 per cent (Campos *et al.*, 2008; Kedzia and Hoderna-Kedzia, 2012).

Proteins (32.8 per cent), essential amino acids (11.5 per cent) and sugars reducing (40.7 per cent) including sucrose, i.e. 3.7 per cent, vitamin C (0.19 per cent), carotene (0.07 per cent) and bioelements i.e. 4.0 per cent, are the average content of the main ingredients in air dried pollen (at 40°C). Baskets for pollen are collected with a special equipment known as pollen traps. As a principle of their activity the pollen basket of field bees that return to the hive. The return route of the bees includes several dividers and bees have to travel a part of the pollen basket is lost and falls into special containers. Pollen traps come in different shapes and sizes and contain enough boxes for outlets, bottom board, slice and top-frame models. They are about 5 mm in height. Bee bread is derived with a special fork scraped from the pebs and then attenuated by a ratio of 1:5 to warm honey. After some days because of its heavy weight the bee bread settles at the bottom of the container and is removed from the honey. The product is tightly sealed and kept in a cool, dark place after filling the jars (Nagai *et al.*, 2004).

1. Carbohydrates and Reducing Sugars: Carbohydrates are a large group of organic substances made up of different carbon-oxygen-hydrogen based chemicals occurring in foods and living tissues and including sugars, starch and cellulose etc. Carbohydrates are the main source of energy for the body. Reducing sugars possess a terminal (free) aldehyde group or ketonic group. eg: maltose, lactose, melibiose, cellobiose etc. Generally the reducing sugar provides the same number of calories as starch and other sugars (Enrique *et al.*, 2019). Starch, gums and cellulose can also be noticed as carbohydrates. According to Todd and Bretherick (1942) it varies by 18.82 to 41.21 per cent while according to Jeanne (1993) it ranges from 25 to 27 per cent. Among carbohydrates the difference between reduction or non-reduction of sugars must be made. It is interesting for bees and people because of the easy digestibility of the sugar reduction. Thus, they are used more effectively by the organism. Honeybees clearly prefer sucrose, glucose, fructose and maltose. It was also observed that bees were fed sucrose for longer periods of time (Dublin, 2015). Only non reduced sugar is present in hand-harvested pollen. When bees collect pollen they add nectar, saliva and other fluids that can transform pollen which lead to an increase in the content of sugar that is very important for living creatures. On an average two-thirds of the non-reduction in pollen balls are inverted by honey bees. The role of glucids is crucial their catabolism means that carbohydrates provide the necessary energy to keep the temperature of their body stable and to allow for flying, drilling and all activities needed for keeping the colony healthy and living. However, carbohydrates can be toxic to bees in very high quantities. Pollen based glucids are used in wax production, feeding and hive management (which are consumed only by larvae, brooders and nurses).

2. Fatty Acids: Fatty acids are the building blocks for the fats. During digestion the body breaks down fats into fatty acids, which can then be absorbed into the blood. Fatty acids is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Fatty acids are major components of cell membrane structure and modulate gene transcription (Norris and Milton, 2013). There are 21 fatty acids found in pollen the main ones being palmitic acid, stearic acid and oleic acid. Lowest quantities of acid are linoleic, linolenic, eicosanoic, lauric, myrtic and lauric etc. Linolenic acid is especially important from a sanitary point of view and to increase bees life span. It has also been concluded that cholesterol are particularly necessary for *Apis mellifera*. Cholesterol is the most common sterol present in nature and it is directly linked to play its role in insects metabolism. Cholesterol is a sloughing hormone and brood stimulant (Svoboda *et al.*, 1986). A



constituent of the bees blood, hemolymph allows insects to resist to very low temperatures in winter. However, it is not synthesized by plants well enough to fulfil insects needs; thus, phytophagous insects must adapt.

3. **Mineral Matter:** Biotic factors like air dust, soil and sprays and pollen origins influence mineral content and concentration. Among the 30 samples from a study in Magneraud (France) there were 97 taxa and the concentration of minerals varies from 1.79 per cent to 3.19 per cent (Feuillet *et al.*, 2008). Phosphorus, calcium and iron are the main elements found in pollen in order of importance. Nitrate, sulphur, copper, sodium, magnesium and most of the other oligoelements are found in several concentrations, which are commonly found in lower amounts (Jeanne, 1993). It has to be noticed that most of the oligo-elements are present in pollen but not all of them in the same pollen (Jeanne, 1993). Conclusions on mineral content are closely linked to pollen species (Jeanne, 1993). The main elements (potassium, phosphorus and calcium) are important in enzymatic metabolism and the nervous system (Maarec, 2015).
4. **Lipids:** Lipids are the compounds that are insoluble in water but are soluble in organic solvents. Lipid serves as the structural building material of all membranes of cells and organelles. They provide energy for living organisms providing more than twice the energy content compared with carbohydrates and proteins on a weight basis (Badriah *et al.*, 2012).

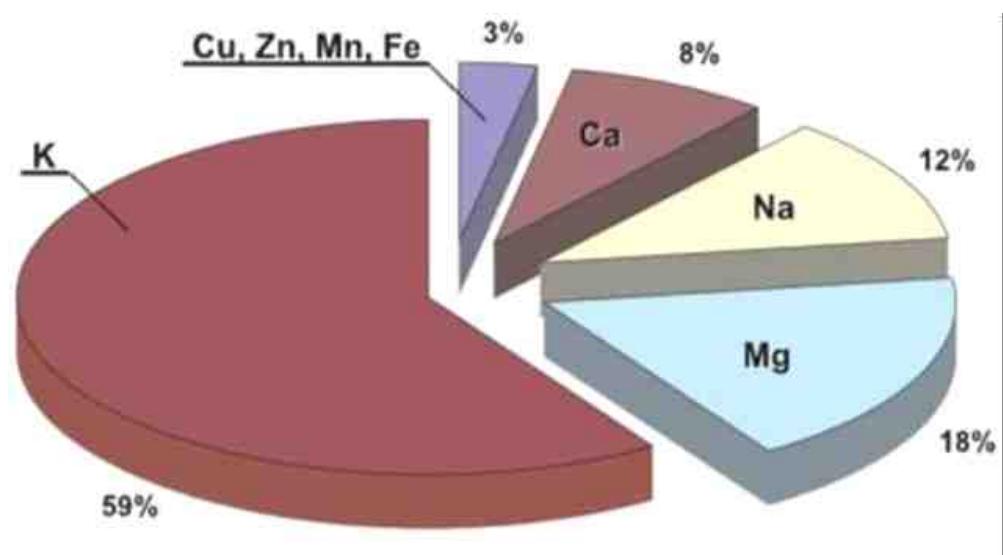
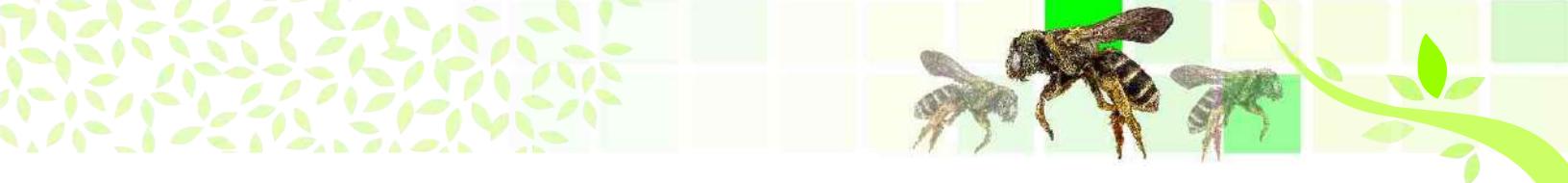


Figure 2: Percentage of Total Minerals Content in Honey Bee-Collected Pollen (Szczesna, 2006)

The activation of important hormones and lubricating food is also responsible for complex lipids (Maarec, 2015). The pollen concentrations detected by Todd and Bretherick (1942) vary from 1.50 to 23.6 per cent and from 7.6 to 22.1 per cent by (Feuillet *et al.*, 2008). The energy value of Pollen is linked to the concentration of lipids. There seems to be a coherence between plants and insects as entomophile plants have more lipid (9 to 14 per cent) than anemophile plants have approximately 2 per cent lipids (Jeanne, 1993). The fact that entomophile plants are more concentrated is related to their visitor because pollinators need lipids.



5. **Proteins:** Proteins are the main components of pollen from the honey bee point of view (Todd and Bretherick, 1942). It promotes the development of their drums and ovaries. Honey bees store pollen in their hive alveoli in order to survive in winter as well as bee bread. The concentration of protein also varies greatly depending on the research and the plants studied. It ranges between 8 to 60 per cent (Todd and Bretherick, 1942; Feuillet *et al.*, 2008; Jeanne, 1993). Protein levels are well preserved in plant species. It means that there is a stable protein concentration in a specific plant species but not in all species. Proteins are amino acid macromolecules which contain a total of 20 different amino acids. Protein intake is one of the key nutrient factors for maintaining independence, primarily in avoiding muscle mass loss and strength, fragility and later comorbidity (Marta *et al.*, 2018).
6. **Amino Acids:** Amino acids are the building blocks of our cellular machinery in the form of proteins and protein complexes. Glenn *et al.* (2019) described the understanding of dietary protein and amino acids and the preventative roles they play with regard to age related demenatis. All the main amino acids are found to contain most pollen. Depending on the dietary disposition of honey bees their effects may be different. Amino acids which play the role of a neuromodulator, deliver neurons to the brain. In the immune system and the preservation of molecular levels in blood, amino acids play a role. The concentration of amino acids is higher and can vary between 3.5 and 24.9 per cent (Zerck, 2013). In 99 per cent of the pollen studied the majority of the amino acids are similar in quantity and the changes in the concentration are due to various plant species. Cook *et al.* (2003) reported that 10 amino acids in a honey bee diet are known to be required. The three most important amino acids (isoleucine, leucine and valine), the less essential (methionine, tryptophane and histidine) and the intermediate ones are classed (arginine, lysine, phenylalanine & threonine).
7. **Vitamins:** Vitamins are the organic substances that are generally classified as either fat soluble or water soluble and minerals are inorganic elements present in soil and water which are absorbed by plants or consumed by animals. It helps to shore up bones, heal wounds and bolster the immune system. They also convert food into energy and repair cellular damage (Bailey *et al.*, 2012). Vitamins are an important part of honey bees. The concentration and composition change according to plants species and the age of pollen. Vitamins stability also varies in the different pollen (Nielsen, 1956). Todd and Bretherick (1942) and Nielsen (1956) analyzed stored pollen of maize, alder and pine. The main vitamins found and in greater quantity were vitamins B1 (Thiamine) and C (Ascorbic acid) and then vitamins B2, B3, B5, B6, B8 (Biotin), A and K. Proportion varying with the source, vitamin B is present in the greatest amount of pollen and has several functions. Thiamine and riboflavin promote hypopharyngeal gland development, increased lifespan, encourage high level nitrogen content and are finally necessary for newly emerged larvae (Herbert *et al.*, 1978).
8. **Nucleic Acids:** The nucleic acids are long chain polymers composed of nucleotides. The nucleic acid forms the chemical basis for the transmission of genetic traits. It serves as sources of energy in the form of ATP (Khedkar and Chopade, 2016).
9. **Water:** 70 per cent of an adult bee weight is water. A honey bee can find water within the surroundings in food and condensed water (Maareec, 2015). The pollen water content depends greatly on the atmosphere. It is a compound harvested; it is not treated as pollen properties (Feuillet *et al.*, 2008). According to Todd and Bretherick (1942) the water content found in pollen balls is 7 to 16.23 per cent. Studies found that the humidity in bee collected pollen is higher than in hand



harvested pollen. Birds with the adding of nectar and saliva may wet the pollen ball but in atmospheric conditions it dries quickly. In fact pollen can lose up to 20 per cent of their weight once dried in the air (Pain *et al.*, 1966).

10. Enzymes: Enzymes are able to break down proteins, carbohydrates, lipids and their supplementation may play a role in the management of digestive disorders (Gianluca *et al.*, 2016).

11. Secondary Metabolites: Secondary metabolites are not important as primary metabolites in highly necessary functions. A secondary metabolite would affect a primary function and others would be involved in fertility and pollen sprouting (Schijlen *et al.*, 2004). Bee collected pollen is made up of flavonoids primarily of significant amounts of polyphenol. The word antioxidant means something opposed to oxidation as the same title indicates. Antioxidant that prevents or inhibits oxygen or peroxide induced reactions. This reduces the harmful effects of free radicals in cells through antioxidants in pollen and can slow down oxidation reactions in the food stuffs (Kroyer and Hegedus, 2001). Pollen contains secondary metabolites, which have received little attention despite their potential significance in honey bee metabolism (Demain and Fang, 2000). Primary metabolites include a variety of compounds.

There are three types of secondary metabolites in plants that have been classified: alkaloids, phenolic compounds and terpenic compounds (Gravot, 2008). Secondary metabolites can also be family specific. Several secondary metabolites are found in the pollens of Senecioneae plants (Decleves, 2014).

1. Lactones sesquiterpenes (may cause toxicity in pollen)
2. Pigments (cause negative influence on honey-bees)
3. Saponines (cause toxicity for honey bees and play a role against fungi)

Saponines have been identified as toxic compound. Bees include sterols in their epidermis membranes to protect against this toxicity. Consequently, some plants can produce unappealing pollen by using toxic secondary metabolites to deter honey bees from foraging on them (Decleves, 2014). Secondary metabolites detected in some pollen include nicotine, anastasine and brassinosteroids which have less attention than alkaloids, phenolic compounds and terpenic compounds. Brassinosteroids because they gather all the hormonal requirements in a very low concentration are considered to be the sixth class of lipid phytohormones as endogenous. They have pleiotropic effects because they affect a range of metabolic actions, including rapid expansion, germination, flora and abiotic stress resistance (Teixeira Zullo and Adam, 2002). Brassinins which promote growth are found in brassinosteroids. Pollen and immature seeds have the highest concentrations, whereas shoots and leaves have the lowest concentrations. Brassinolide (steroidal lactone) is a plant growth promoter that is biologically active (a steroidal hormone). Insects and larvae are affected by brassinosteroids.

Depending upon the concentration it stimulates cell division, elongation and morphology changes (Teixeira Zullo and Adam, 2002). Pollen also includes microorganisms involved in the fermentation process such as spore germs, fungus and bacteria (Pain *et al.*, 1966). Pseudomonas which play a role in anaerobic development, lactobacillus and osmophilic yeast and saccharomyces play important nutritive and appetising roles all intervene in stored pollen. Finally pollen contains waxes, oils, resins and hormones that stimulate development (Todd and Bretherick, 1942). Some unidentified materials have been found in pollen shells, owing resistance to digestive processes and weathering.



Chapter-3

HEALTH BENEFITS

Health Benefits of Bee Pollen:

Humans use bee pollen as an energy source in their diets and for athletic performance. Bee pollen has a nutritional value that is comparable to or higher than that of dried legumes due to its high content of protein, fat and minerals (particularly Ca, Mg, Fe and P). “Among vitamins, the content of vitamin B-5 (pantothenic acid) and vitamin B-3 (nicotinic acids) are near to those of beef, vitamin C (ascorbic acid) is equivalent to that of vegetables like lettuce and tomatoes and Vitamin B-2 (riboflavin) is comparable to that of skimmed milk powder” (Linskens and Jorde, 1997). “Prostatitis, stomach ulcers, prevention and recovery of high altitude sickness syndrome are all treated with bee pollen as a complementary and alternative medicine” (Linskens and Jorde, 1997). “Antimicrobic, chemopreventive, antioxidant, hepatoprotective, anticarcinogenic, antiatherosclerotic, anti-inflammatory, antiallergic and immunomodulatory activities have been suggested for bee pollens in the literature” (Komosinska-Vassev *et al.*, 2015; Denisow and Denisow-Pietrzyk, 2016). Because of its possible medicinal and nutritional advantages bee pollen is a highly useful apitherapeutic commodity that is highly valued by natural medicine. “Antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anticancer, immunostimulating and local analgesic efficacy are both shown through empirical proof” (Katarzyna *et al.*, 2015). Bee pollen also expedites the process of granulation in burn wound healing (Almaraz-Abarca *et al.*, 2004; Kroyer and Hegedus, 2001).

- 1. Antioxidant Activity:** Pollen comprises phenolic acids like vanillic acid, protocatechuic acid, gallic acid and p-coumaric acid as well as “flavonoids like hesperidin, apigenin, luteolin, rutin, kaempferol, quercetin and isorhamnetin” which have antioxidant properties. Electrophiles are inactivated by these molecules which also serve as free radical scavengers and reactive oxygen species (Bonvehi *et al.*, 2001; Pascoal *et al.*, 2014).
- 2. Antimicrobial Activity:** Antimicrobial properties of bee pollen are well known and can be due to the action of the enzyme glucose oxidase used in honey bee secretion as well as plant phenolics and flavonoids compounds (Denisow and Denisow-Pietrzyk, 2016; Fatrcova-Sramkova *et al.*, 2016). The action of phenolic compounds from bee pollen extracts against gram-positive and gram-negative pathogenic bacteria, microscopic fungi and yeasts has also been noted by researchers (Baltrusaityte *et al.*, 2007; Kacaniova *et al.*, 2012).



3. **Anti-inflammatory Activity:** The action of flavonoids, phenolic acids, phytosterols and flavouring substances like anethole an inhibitor of the NF-KB pathway has been linked to anti-inflammatory effects in bee pollen that have been compared to those of traditional non steroidial anti-inflammatory medications (Middleton, 1998; Choi, 2007). Specific benefits include the ability to reduce swelling caused by cardiovascular and renal diseases (Yakusheva, 2010) protect the liver from carbon tetrachloride-induced damage (Yildiz *et al.*, 2013), and suppress prostate inflammation and hyperplasia (Yildiz *et al.*, 2013; Yakusheva, 2010). Positive effects on prostatic conditions have been also ascribed to antiandrogen actions (Rzepecka-Stojko *et al.*, 2012).
4. **Anticancer Activity:** Bee pollen has been found to have anticancer properties in several experiments which are most likely due to its antioxidant and antimutagenic properties (Denisow and Denisow-Pietrzyk, 2016). The steroid fraction of a chloroform extract from *Brassica campestris* bee pollen has shown strong cytotoxicity on human prostate cancer PC-3 cells, associated with stimulation of TNF-a secretion and apoptosis induction (Wu and Lou, 2007).
5. **Anti-atherosclerotic and Anti-diabetic Properties:** Bee pollen has been shown to have anti-atherosclerotic and cardioprotective properties and it has been used effectively in patients that have not responded to traditional medications (Polanski *et al.*, 1998). Hypolipidemic activity confirmed by pharmacological studies conducted on rats and rabbits has been ascribed to the presence of unsaturated fatty acids, especially the ω-3, α-linolenic acid and to phospholipids and phytosterols (Komosinska-Vassev *et al.*, 2015). α-linolenic acid is a precursor of prostaglandin-3a that is considered a major inhibitor of platelet aggregation (Denisow and Denisow-Pietrzyk, 2016). Ghoshal and Saoji (2013) have found the presence of antidiabetic compounds in pollen grains such as steroids and alkaloids in the pollen of *Catharanthus roseus*, saponins, flavonoids, sugars and tannins in *Momordica charantia*, sugars, flavonoids and sterols in *Butea monosperma* and alkaloids and tannins in *Syzygium cuminii* suggesting therapeutic possibilities for bee pollen as a hypoglycemic agent.
6. **Antiallergic Activity:** Evidence of bee pollen antiallergic activity has been published in the literature including avoidance of IgE binding to their high-affinity receptor Fc + RI, inhibition of histamine release from mast cells and basophil degranulation (Ishikawa *et al.*, 2008; Moita *et al.*, 2014). Flavonoids, steroids and volatile oil compounds are involved in immune suppressive activities.
7. **Nutritional Values:** Bee pollen has been used as a food supplement at times of regeneration in cases of malnutrition, asthenia and to increase physical and mental ability or strengthen the immune system. Experiments on animals have shown that the administration of bee pollen prolongs life span, promotes weight gain, increases plasma hemoglobin levels and provides tissues with vitamin C and Mg (Khalil and El-Sheikh, 2010; Attia *et al.*, 2011). These virtues may be related to a complex of active substances including amino acids, vitamins, polyphenols, carotenoids, phytosterols and minerals (Denisow and Denisow-Pietrzyk, 2016).
8. **Health Hazards:** Health hazards associated with bee pollen use can arise from the presence of pollutants such as bee venom, heavy metals, pesticides, mycotoxins and bacteria on occasion (Denisow and Denisow-Pietrzyk, 2016). Moreover, bee pollen derived from *Echium vulgare* and *Symphytum officinale* may contain dangerous levels of pyrrolizidine alkaloids with hepatotoxic properties (Kempf *et al.*, 2010). As known, pollen is highly allergenic and consequently complications or anaphylaxis due to bee pollen use have already been reported so sensitivity tests are highly recommended for individuals before use (Jagdis and Sussman, 2012).



Chapter-4

BEE POLLEN AND PROPOLIS: A Remedy for Covid-19

Bee Pollen and Propolis as a Remedy for COVID-19 (SARS-CoV-2):

Bee Pollen:

“Most of the compounds found in bee pollen particularly polyphenols have promising activity in opposition to CoVhs which include SARS-CoV and MERS” (Chen *et al.*, 2006; Yi *et al.*, 2004). Eight various types of phenolic compounds have been recognized in bee pollen by liquid chromatography analysis. “These compounds can be divided as: flavonoids represented by means of flavonols, chalcones, flavanols, flavone, flavanones, anthocyanidin and isoflavones; and non-flavonoids compounds along with phenolic acids” (Denisow and Denisow-Pietrzyk, 2016; Thakur and Nanda, 2020). “Bee pollen has higher concentrations of phenolic phytochemicals such as quercetin and kaempferol as well as their glycosides derivatives” (Rzepecka-Stojko *et al.*, 2015).

As per Yi *et al.* (2004) “entry of SARS-CoV into Vero E6 cells in vitro was inhibited by quercetin”. “The concentration required to inhibit 50% of SARS-CoV (EC₅₀) was 83.4 µM, which is low value as this compound within the bee pollen is found in high concentration (~60 µmol/g of quercetin is found in pollen of *Apis mellifera L.*)” (Carpes *et al.*, 2013). “Quercetin-3β-galactoside, a glycoside derived from quercetin frequently found in bee pollen (Rzepecka-Stojko *et al.*, 2015) binds to SARS-CoV 3CL^{pro} and inhibits its proteolytic activity with an IC₅₀ of 42.79 ± 4.95 µM”. “This inhibitory effect of SARS-CoV 3CL^{pro} relies upon the hydroxyl group of quercetin. Gln189 residue is present on the active site of 3CL^{pro} where hydroxyl group of quercetin forms a hydrogen bond with Gln189 residue” (Chen *et al.*, 2006). As SARS-CoV-2 3CL^{pro} keeps the same Gln189 residue in its active site, it is suggested that the main protease of corona virus may also be susceptible to the inhibitory action of quercetin and its derivatives. As per Rehman *et al.* (2020) “it is anticipated that a hydrogen bond with Gln186 is formed with quercetin on active site of SARS-CoV-2 3CL^{pro}, which results in low binding energy (-7.5 kcal/mol) and consequent high binding affinity ($3.2 \times 10 \text{ M}^{-1}$)”. As per Li *et al.* (2020) and Brockway and Denison (2004) 3a channel protein of corona virus is inhibited by kaempferol and its glycosides analogs. A cation-selective channel is formed by 3a protein which becomes expressed in the infected cell and is involved in virus release mechanism. “Virus release is also inhibited by the drugs which inhibit the ion channels and such drugs can become a source for the development of therapeutic antiviral agents against SARS-CoV-2”.



"In oocytes of *Xenopus laevis* (African clawed frog) with expressed SARS-CoV 3a protein, kaempferol weakly inhibited Ba²⁺ sensitive current" (Schwarz *et al.*, 2014). Schwarz *et al.* (2014) reported that kaempferol glycoside juglanin was most effective against SARS-CoV 3a protein (IC_{50} 2.3 μ M). "A study found that on SARS-CoV-2 3CL^{pro}, Kaempferol may additionally act showing binding energy and binding affinity of -7.8 kcal/mol and 5.2×10^5 M⁻¹. It forms two hydrogen bonds, one with the Gln189 residue which is important to the catalytic activity of 3CL^{pro}" (Rehman *et al.*, 2020). "Moreover, quercetin and kaempferol exhibit inhibitory effects on inflammatory (IL-8, IL-6, iNOS, IFN- γ , NF- κ B, COX-2) (Pan *et al.*, 2010) and oxidative stress (RNS, LOX-1, ROS) mediators" (Banjarnahor and Artanti, 2014). "It indicates that they might additionally prevent immunologic complications related to COVID-19 including the macrophage activation syndrome which ends up in a potentially fatal "cytokine storm" and acute respiration distress syndrome" (Biancatelli *et al.*, 2020). "When immunologic complications like cytokine storm occur the antiviral treatment should be combined with anti-inflammatory treatment" (Zhai *et al.*, 2020; Biancatelli *et al.*, 2020)."Bee pollen that's the bee product with excessive quantities of kaempferol, quercetin and its derivates can be a promising alternative to fight towards COVID-19"(Rzepecka-Stojko *et al.*, 2015).

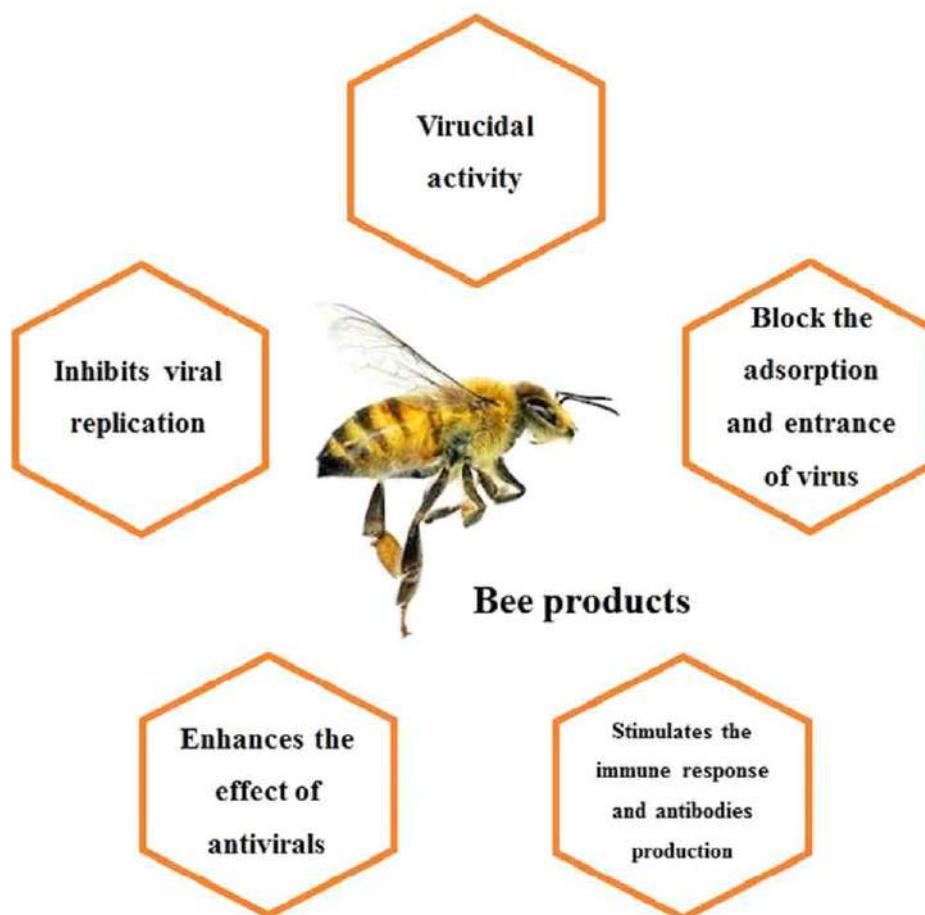


Figure 3: Bee Products Effect that can be Harvested Against the Novel Corona Virus (SARS- CoV-2) (Lima *et al.*, 2020)



Table 1: *Cistus* L. Bee Pollen Phenolic Composition (Guler and Kara, 2020).

Standards($\mu\text{g/g}$)	<i>Cistus</i> Polen $\mu\text{g}/100\text{g}$
Hydroxybenzoic acids	
Syringic acid	97.40
Protocatechuicacid	Not detected
p-OHBenzoicacid	4207.50
Gallicacid	8528.00
Catechin	
Epicatechin	Not detected
Catechin	4691.70
Hydroxycinnamicacids	
t-Cinnamicacid	61.30
p-Coumaricacid	2838.50
Ferulic acid	2844.90
CAPE	851.80
Caffeicacid	Not detected
Flavonols	
Myerecetin	Not detected
Rutin	4599.74
Flavanones	
Pinocembrin	106.44
Chyrsin	1426.12
Hesperetin	Not detected
Isoflavons	
Daidzein	Not detected
Flavones	
Luteolin	Not detected
Stilbandsand Lignans	
Resveratrol	Not detected



Guler and Kara (2020) revealed the *in silico* effects on CoV-2 Spike glycoprotein Human ACE-II complex of *Cistus L.* bee pollen and inhibition was studied with the eleven flavonoids as important substances. As per Sargin and Selvi (2016) in Turkey the genus *Cistus L.* (Cisteceae) commonly known as Laden has five species which are *Cistus creticus L.*, *Cistus laurifolius L.*, *Cistus monspeliensis L.*, *Cistus parviflorus Lam.* and *Cistus salvifolius L.* It is mostly found in the Marmara, Black Sea and Mediterranean regions. Historically, a few species of *Cistus* has been used as herbal tea amongst people to cure digestive troubles and colds. As per Papaefthimiou *et al.* (2014) *Cistus creticus* had secondary metabolites especially phenolic compounds in leaf mesophilia and glandular trichomes. Secondary metabolites have antibacterial, antioxidant, anti-inflammatory, anti-fungal and anti-cancer properties. Gallic acid (8528.00 g/g), catechin (4691.70 g/g) and rutin (4599.74 g/g) phenolic compounds were reported to be highest in bee pollen (Guler and Kara, 2020). The most common phenolic compound found in *Cistus* bee pollen was kaempferol. The phenolic composition of pollen differs depending on geographic conditions, plant type, harvesting period and dominant pollen type. Catechin, pinocembrin, chrysin and caffeic acid phenethyl ester compounds effectively inhibit the CoV-2 Spike glycoprotein-Human ACE-II complex, according to his findings. These compounds can be checked in the clinic and used to treat Covid-19 (Guler and Kara, 2020). “Bee pollen a bee product rich in quercetin, kaempferol and its derivates” (Rzepecka-Stojko *et al.*, 2015) can be a promising alternative to combat COVID-19 as well as other bee products propolis and royal jelly. Propolis is commonly used in the prevention and treatment of COVID-19 (500 mg/day is equivalent to 30 drops of propolis extract (11 per cent w/v of dry matter), 3 to 4 times a day, diluted in around 100 ml of water) but it may also be used in more extreme cases of COVID-19 with dosages greater than 500 mg/day.

Few studies have reported bee pollen antiviral activity: in unspecified pollen (Komosinska, 2015), for the pollen flavonoid kaempferol (Humphery and Busath, 2019) and for a 1:1 mixture of pollen and manuka honey (Teupa, 2018). Quercetin a flavonoid contained in pollen has been shown to have antiviral activity against influenza virus (Banskota *et al.*, 2001).

“Latest molecular docking studies confirmed that 5,7,3',4' tetrahydroxy-2-(3,3-dimethylallyl) isoflavone, myricitrin and methyl rosmarinate could inhibit SARS-CoV-2 3CLpro and could be used as anti-COVID-19 drug compound” (Qamar *et al.*, 2020). Quercetin (IC₅₀) = 73 M, epigallocatechin gallate (IC₅₀) = 73 M and gallocatechin gallate (IC₅₀) = 47 M all inhibited 3CLPro effectively.

“GCG had a binding energy of -14 kcal mol (-1) to the active site of 3CL(pro) in molecular docking experiments and the galloyl moiety at the 3-OH position was necessary for 3CL(pro) inhibition action” (Nguyen *et al.*, 2012). According to Ryu *et al.* (2010) “the apigenin moiety at position C-3 of flavones appeared to be more powerful”. The researchers discovered that amento flavone (IC₅₀) = 8.3 μM, apigenin (IC₅₀) = 280.8 μM, luteolin (IC₅₀) = 20.2 μM and quercetin (IC₅₀) = 23.8 μM as well as the ethanol extract of *Torreya nucifera* leaves which is commonly used as a medicinal plant in Asia (62 per cent), have a 3CL(pro) inhibitory effect. Previous research showed quercetin-3-beta-galactoside to be a potent protease inhibitor and a structure-activity relationship for the new compounds was proposed:

1. The bioactivity of the derivatives is reduced when the 7-hydroxy group of the quercetin moiety is removed.
2. The sugar moiety is acetoxylated which eliminates the inhibitory effect.
3. The addition of a significant sugar substituent to quercetin's 7-hydroxy can be tolerated.
4. The inhibitor efficacy is unaffected by replacing the galactose moiety with other sugars.



Figure 4:

Flowers from Representative *Cistus* Plants. Three Species of *Cistus* Flowers are shown as an Example of White (*Cistus salviifolius*, Leucocistus Subgenus, A); Purple (*Cistus incanus*, *Cistus* Subgenus, B); and Dark Red Spotted White Flowers (*Cistus ladanifer*, Leucocistus Subgenus, C). (Barrajon-Catalan *et al.*, 2016)

Propolis:

“Propolis also called bee glue is a sticky waxy substance made up of bee salivary secretions, bee wax and resinous sap found in the bark and leaf-buds of specific plants” (Boisard *et al.*, 2020). Based on the collected local flora it comes in grey, brown, red and black colours. The terms propolis and “polis” are Greek words that mean “in front of or at the entrance to” and “group or city,” respectively. “Propolis is a hive-protecting agent that bees use to defend and rebuild their hives” (Anjum *et al.*, 2019). It is a unique product of a complex combination that comprises more than 420 chemical components. Nonetheless, depending on its botanical and geographical sources as well as the time of harvesting its composition and biological activities differ significantly. Propolis is rich source of oxyprenylated phenylpropanoids secondary metabolites from plants, bacteria and fungi, namely 7-isopentenyloxycoumarin, boronic acid, 4-geranyloxyferulic acid and auraptene. 4-geranyloxyferulic acid and auraptene exist in raw Italian propolis at high concentrations: 107.12 and 145.37 µg/g of dry propolis, respectively.



Flavonoids, a huge group of phenolic compounds are found in Italian propolis and they are divided into many groups including flavanones (e.g., naringenine, 4.4 mg/g), flavonols (e.g., galanin, 0.9 mg/g), flavones (e.g., apigenin, 1.7 mg/g), catechins (expressed as (+) -catechin 0.4 mg/g, tannins (e.g., gallic acid 8.4 mg/g) and caffeic acid and its esters (expressed as caffeic acid, 9.2 mg/g) (Genovese *et al.*, 2017).

“Artepillin C (38.6 mg/g), kaempferide (12.6 mg/g) and coumaric acid (10.6 mg/g) are the most abundant flavonoids in ethanolic extracts of Brazilian propolis” (Ueda *et al.*, 2013). Polyphenols (e.g., phenolic acids and aromatic esters), terpenoids, phenolic aldehydes, ketones enzymes (e.g. α- and β-amylase), vitamins (e.g. thiamin (B1), riboflavin (B2) and pyridoxine (B6), ascorbic acid (C), tocopherol (E), minerals, essential oils, fatty acids, alcohol and β-steroids are all important components of propolis (Boisard *et al.*, 2020). Western honey bees (*Apis mellifera L.*) make propolis from resins collected from various plant organs and with which they mix bee wax. The word “propolis” comes from Greek: “pro” means “in front of/for” and “polis” means “city” so it means “in front of the city.” “Bees use propolis as a construction agent in their hives, sealing gaps and cracks, fixing combs and reinforcing the combs thin borders” (Ghisalberti, 1979). “A coating of propolis called the “propolis envelope” is applied by feral bees to the inside of tree cavities” (Seeley and Morse, 1976). “Propolis serves as a chemical barrier against microorganisms as well as an embalmer for larger, dead intruders (insects & small animals) that have died in the hive and are too large for the bees to remove” (Ghisalberti, 1979). “Propolis beneficial medicinal, therapeutic properties have been known by humans for millennia; historical documents indicate that it was used by the ancient Egyptians, Romans and Greeks” (Crane, 1999). “It is still widely used as a home remedy in many countries around the world as well as a component of food additives, cosmetics and over the counter medications” (de Groot, 2013; Suarez *et al.*, 2005). The chemical composition of propolis determines its biological activity which is dependent on the source plant from which bees extract the resin. Propolis has been classified into a variety of chemical groups based on the plant source. Understanding the chemical diversity of propolis is crucial to propolis research.

Propolis Extraction from Hives:

Commercial Traps: Propolis traps are sold by the big commercial bee keeping supply companies. These are usually dense plastic sheets with a series of 1.6 mm grooved slits running the length of the sheet. “This is the width at which honey bees are encouraged to deposit more propolis and less wax in order to close the opening” (Crane, 1990). The chemical composition of propolis determines its biological activity which is dependent on the source plant from which bees extract the resin. Propolis has been classified into a variety of chemical groups based on the plant source. Understanding the chemical diversity of propolis is crucial to propolis research.

1. “Position the propolis trap directly over the top frames of the colonies upper most box (super) and cover with a regular colony lid” (Crane, 1990).
2. “By increasing air flow and light through the trap, trap success can be improved” (Crane, 1990; Krell, 1996). Place a wooden rim with holes drilled into its sides over the propolis trap and under the outer cover to accomplish this. Using a migratory cover (a flat cover without an overhang to cover the gaps in the rim) aids this method even further. While this extra step is not needed it will help you gather more resin.
3. “It is important to remember that depending on genetics, climate and colony strength, the amount and quality of propolis collected can differ greatly between colonies” (Butler, 1949; Wilson *et al.*, 2015). In a couple of weeks, a solid high resin-collecting colony may fill a trap with propolis. “Since there is a genetic aspect to the



degree of propolis collection demonstrated by bees, other colonies will never fully close all gaps or will use mostly wax to seal the gaps" (Manrique and Soares, 2002).

4. "It is better to freeze the traps before harvesting the propolis so that the propolis becomes hard and brittle" (Krell, 1996). It can then be scraped or kicked out of the traps.

Non-Commercial Propolis Traps: "Propolis can be collected using a variety of materials" (Krell, 1996). The key is to ensure that the substance cannot be chewed away by the bees and that the gaps are large enough to facilitate resin deposition.

1. Mesh (burlap) bags such as those used to store corn, potatoes and other crops are one viable alternative. These bags when doubled over and set on top of the colony in the same way that commercial traps are placed perform exceptionally well. Landscape cloth also can be used.
2. Freeze the cloth before extracting the propolis with commercial traps. The propolis would be released from the gaps by rolling the fabric on a hard surface.

Hive Scrapings: Scraping propolis from the frame rests, frame edges and bottom boards or insides of boxes is the most popular way to harvest propolis in an apicultural environment (Krell, 1996). This is usually done at the end of the season to clean up the boxes so they can be used again the next year and it can easily produce a significant quantity of propolis. Scrapings can contain propolis from many seasons and the effect of age on propolis quality is uncertain. More research is required to see whether propolis antimicrobial properties deteriorate over time.

Chemical Composition of Propolis: "The chemical composition of propolis contains a large number of phenolic compounds. Polyphenols are a class of widely distributed and chemically diverse secondary metabolites synthesised in plants at various stages of growth" (Steinmann and Ganzenra, 2011). At least one aromatic ring with one or more hydroxyl functional groups is found in polyphenols. Flavonoids are the most abundant group of phenolic compounds, with structures based on a C6-C3-C6 skeleton. "They are classified into several groups based on the oxidation state of the central heterocyclic ring" (Veitch and Grayer, 2008). Chalcones, flavones, flavonols, flavanones, isoflavonoids, anthocyanidins and flavanols are among them (catechins and tannins). Non-flavonoids consist simple phenols, phenolic compound (galangin, chrysin, quercetin, kaemferol and p-coumaric acid), phenolic acids, coumarins, xanthones, stilbenes, lignins and lignans. "Benzoic acid derivatives which have a C6-C1 skeleton and cinnamic acid derivatives which have a C6-C3 skeleton, are the two types of phenolic acids" (Veitch and Grayer, 2008).

Characteristics and Bioactive Composition of Propolis:

Characteristics: Propolis is a lipophilic, stiff and brittle substance that when heated becomes flexible, pliable, gummy and extremely sticky. It has a distinct and friendly aromatic odour and its colour ranges from yellow green to red to dark brown depending on the source and age. It can also vary in colour from yellow to dark brown, depending on the origin of the resins.

Composition: Propolis is a complex mixture of plant-derived and bee-released compounds. In general, raw propolis is made up of about 50 per cent resins, 30 per cent waxes, 10 per cent essential oils, 5 per cent pollen and 5 per cent other organic compounds (Burdock, 1998). More than 300 constituents have been identified in various samples (Marcucc, 1995) and new ones are still being discovered as new forms of propolis are chemically characterised (Bankova *et al.*, 2000). The proportions of the different substances found in propolis vary depending on where and



when it was collected. Many analytical methods have been used to separate and identify propolis constituents and the substances discovered fall into one of the following chemically related group: cinnamic alcohol and cinnamic acid and its derivatives; benzoic acids and derivatives, sesquiterpene and triterpene; hydrocarbons; polyphenols; benzaldehyde derivatives; other acids and respective derivatives; ketones, alcohols and heteroaromatic compounds; sesquiterpene alcohols and terpene and their derivatives; minerals; sterols and steroid hydrocarbons; aliphatic hydrocarbons sugars and amino acids (Walker and Crane, 1987).

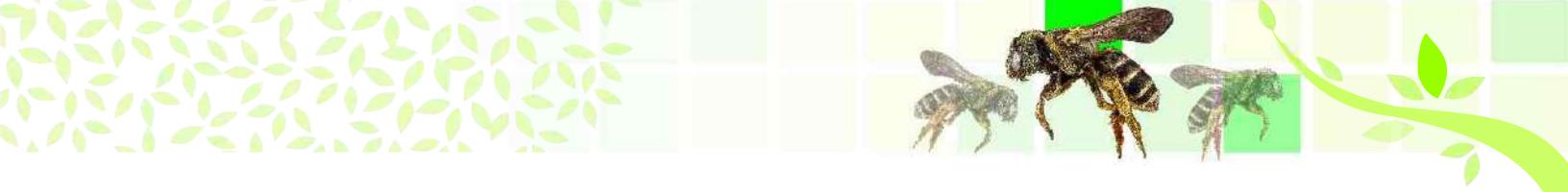
Comorbidities in COVID-19 Patients as well as Evidence that Propolis can help to Mitigate their Impact:

Cancer: COVID-19 finds cancer to be a relevant comorbidity factor. Patients with cancer have a 3-4 times greater chance of developing serious COVID-19 disease than those without. Furthermore, during a Corona virus pandemic the hospital atmosphere can interfere with and interrupt cancer patients care. Patients who are experiencing symptoms may decide not to visit a clinic or hospital to see if they have cancer (Patel, 2016). Alternative treatments may be able to help COVID-19 patients delay cancer or reduce the effects of cancer and cancer care. Propolis has the ability to be used as a cancer complementary therapy. “It has been shown to be effective in the treatment of cancers of the bladder, blood, brain, breast, colon, head, neck, kidney, liver, pancreas, prostate and skin” (Frion-Herrera *et al.*, 2020). Propolis extracts have been found to suppress tumour cell growth *in vitro* and *in vivo* as well as angiogenesis indicating that they may be used to create new anticancer drugs (Song *et al.*, 2002). “Cinnamic acid (Akao *et al.*, 2003), CAPE (Wu *et al.*, 2011), quercetin (Orsolic *et al.*, 2004) and chrysin (Sawicka *et al.*, 2012) have all been shown to inhibit cancer cell development.” Propolis and its components have a distinct cytotoxicity in liver cancer, melanoma and breast cell carcinoma cell lines than they do in normal cells.

Cardiovascular and Hypertension Disease: COVID-19 considers cardiovascular and hypertension disease to be important comorbidities (Cook, 2020). It is used in traditional medicine in Cameroon to treat a variety of illnesses including high blood pressure (Zingue *et al.*, 2017). For its health benefits including cardiovascular safety, propolis has been commonly used as a dietary supplement. “Consumption of propolis improved essential blood parameters in a human indicating that it may help reduce the risk of cardiovascular disease” (Yuan *et al.*, 2019).

Obesity: In COVID-19 patients, obesity is a significant comorbidity and indicator of increased mortality. “Obesity and SARS-CoV-2 also cause inflammation making SARS-CoV-2 infection worse in obese people” (Michalakis and Ilias, 2020).

Old Age: “Chronic inflammation marked by systemically elevated levels of pro-inflammatory cytokines is more common in the elderly and can lead of a cytokine storm a major cause of COVID-19 mortality” (Gubernatorova *et al.*, 2020). Propolis contains antioxidants which can help to slow or stop the ageing process. CAPE a propolis part helped *Caenorhabditis elegans* a popular model organism for ageing research live longer (Havermann *et al.*, 2014). Consumption of propolis shielded elderly (human) subjects from cognitive loss when they were exposed to high altitudes. TGF-1 and IL-10 levels in the blood were substantially higher in propolis treated elderly people possibly reducing inflammation and protecting them from cognitive decline. “In men treated with propolis the activity of superoxide dismutase (SOD) a main antioxidant increased whereas malondialdehyde an oxidative stress marker, decreased” (Jaspica *et al.*, 2007). Senescence is linked to the angiotensin system which is essential for SARS-CoV-2 invasion of host cells. One explanation that SARS-CoV-2 causes substantially higher mortality in older patients may



be because they have a higher number of senescent lung cells which are a soft target for viral infection and may facilitate viral replication. Senolytic drugs may be useful in helping the elderly survive COVID-19. “Quercetin a part of propolis that has been suggested as a COVID-19 drug has senolytic activity” (Sargiacomo *et al.*, 2020).

Diabetes: Diabetes is a common comorbidity associated with a high mortality rate in critically ill COVID-19 patients (Fuliang *et al.*, 2020). “Given the connection between diabetes and inflammation as well as the fact that flavonoids the main bioactive components of propolis protect against free radicals and other pro-oxidative compounds, it's possible that consuming propolis can lower diabetes risk” (Vinayagam and Xu, 2015). “Since it can help prevent inflammation and diabetes, Brazilian propolis has become popular as a safe dietary supplement in various parts of the world” (Tiveron *et al.*, 2016).

Kidney Diseases: COVID-19 poses a significant risk to patients who have comorbidities including renal or hepatic dysfunction (D'Marco *et al.*, 2020). SARS-CoV-2 often targets the kidney. COVID-19 patients have a higher chance of kidney failure and as a result many COVID-19 patients have renal dysfunction. COVID-19 patients with chronic kidney disease and those on hemodialysis have a higher mortality rate. Propolis has been shown to have anti-kidney disease properties. In animal models, propolis treatment prevented nephropathy (Bhadauria, 2012).

Bacterial Infection: “In COVID-19 bacterial infection is a common complication” (Wang *et al.*, 2019). Propolis has a long history of use for its antibacterial properties and it may be able to support COVID-19 patients with bacterial infections. “Propolis healing properties are mentioned in the old testament and Hippocrates recommended it for the treatment of sores and ulcers in Ancient Greece” (Cushnie and Lamb, 2005). Propolis has been prized for its antibacterial properties in Russia and other Eastern European countries for decades. Galangin, pinocembrin, rutin, quercetin and naringenin as well as CAPE increase the permeability of bacterial membranes which may explain their antimicrobial properties (Cornara *et al.*, 2017). Propolis key mechanism of action according to De Campos *et al.* (2019) is bacterial cell rupture and lysis.

Mechanism: There is a considerable good amount of literature supporting quercetin's antiviral properties in both *in vitro* and *in vivo* studies. In cultured cells, quercetin inhibits many respiratory viruses (Debiaggi *et al.*, 1990). “Many serotypes of rhinovirus, echovirus (types 7, 11, 12 and 19), coxsackievirus (A21 and B1) and poliovirus (type 1 Sabin) are inhibited by quercetin for their cytopathic symptoms at a low inhibitory concentration of 0.03 to 0.5 µg/ml in Helaor WI-38 cells” (Itsuka *et al.*, 1982).

Effect on SARS-CoV-2: “Quercetin has been studied for its antiviral properties against many members of the Corona viridae family and quercetin provides great promise as a potential drug in the clinical treatment of SARS according to” (Yi *et al.*, 2004). “SARS Coronavirus is a single-stranded RNA virus with 29,700 nucleotides that uses ribosome sites to encode two replicase glycoproteins, PP1a and PP1b that facilitate viral replication” (Rota *et al.*, 2003; Marra *et al.*, 2003). “3C-like protease (3CLpro) is involved in the lytic release of its replicates after these precursor glycoproteins have been synthesised” (Snijder *et al.*, 2003). With an IC₅₀ of $42.79 \pm 4.95 \mu\text{M}$, quercetin-3-galactoside binds to SARS-CoV 3CL protease and inhibits its proteolytic action. “This inhibitory effect on 3CLpro is due to quercetin hydroxyl group which recognises Gln189 as a critical site on 3CLpro for quercetin binding as shown by molecular modelling and the Q189A mutation” (Chen *et al.*, 2006). “Quercetin was also discovered to be a compound capable of blocking SARS-Corona virus entry into Vero E6 cells with an EC₅₀ of 83.4 M and low cytotoxicity (CC₅₀ 3.32 mM)” (Yi *et al.*, 2004). “SARS-CoV-2 the virus responsible for the COVID-19 disease outbreak in 2020 (Zhou *et al.*, 2020), belongs to the genus Beta Corona virus and subgenus Sarbecovirus and it is



thought to infect type II pneumocytes entering through the angiotensin converting enzyme II receptor similarly to SARS-CoV” (Lu *et al.*, 2019). “The Gln189 site of SARS-CoV 3CL pro which was previously known as the binding site for the hydroxyl groups of quercetin and its derivatives is still present in SARS-CoV-2 protease 3CL” (Chen *et al.*, 2006). Despite the scope and depth of antiviral *in vitro* and *in vivo* studies into quercetin immunomodulatory effects further research is needed to confirm quercetin’s inhibitory activities on SARS CoV-2 virus entry RNA polymerase.

Doses:

“A placebo elixir and Chizukit (a common over-the-counter medication containing *Echinacea* extract 50 mg/ml (*Echinacea purpurea* and *Echinacea angustifolia*) 50 mg/ml of propolis extract and 10 mg/ml of vitamin C for respiratory tract infection) were used in a randomised, double-blind, placebo-controlled clinical trial with 430 children (1-5 years old) in Israel and found results” (Cohen *et al.*, 2004).

Another clinical trial for asthma care in adults was conducted (Khayyal *et al.*, 1993). TNF-a, ICAM-1, IL-6, IL-8 and a 3-fold increase in the “protective” cytokine IL-10 were all reduced in the analysis which used a propolis water extract. The levels of prostaglandins E2, F2a and leukotriene D4 were also reduced significantly (Khayyal *et al.*, 1993). Berretta *et al.* (2017) studied a lot of them and found that the most popular dose for adults was 500 mg per day. “In the case of EPP-AF®, clinical evidence to date supports daily doses of 375-500 mg of propolis however, non-clinical studies showed that much higher doses can be tolerated and may be beneficial” (Waldesch *et al.*, 2003). The 500 mg/day dosage will be equal to 30 drops of propolis extract (with 11 per cent w/v of dry matter), 3 to 4 times a day, diluted in around 100 ml of water or 3 to 4 units/day of propolis capsules or tablets. 30 drops or one capsule are normally taken once a day for prevention. The therapeutic activities of individual bioactive compounds in propolis have recently been the subject of many drug targeting studies. Flavonoids make up the bulk of the bioactive substances in propolis that have been studied. Chrysin (5,7-dihydroxyflavone) is a flavonoid found in mushrooms, flowers (such as blue passion flower) and bee products (e.g., honey). “It has anti-proliferative, anti-inflammatory and neuroprotective its antioxidant properties” (Havermann *et al.*, 2014).

The main bioactive constituents of propolis are essential/volatile oils which contribute to its distinct aroma. They also play a role in propolis powerful antimicrobial, antioxidant and anticancer properties. Bachevski *et al.* (2020) revealed that SARS researchers have been paying special attention to quercetin a flavonol contained in propolis since quercetin in combination with vitamin C has been found to be an important amino peptidase inhibitor (Syed and Saleem, 2004). *In vitro* quercetin and its derivatives inhibit the key protease of SARS-CoV-1 and MERS-CoV. Quercetin also affects the cellular response to unfolded proteins (UPR). Since Corona viruses may use the UPR to complete their entire replication cycle quercetin’s modulation of this pathway may have anti-Coronavirus effects (Polansky and Lori, 2020). *In vitro* quercetin and its derivatives inhibit the key protease of SARS-CoV-1 and MERS-CoV. Quercetin also affects the cellular response to unfolded proteins (UPR). Since Corona viruses may use the UPR to complete their entire replication cycle, quercetin’s modulation of this pathway may have anti-Corona virus effects (Polansky and Lori, 2020).

Targeting downstream effectors, such as p21-activated kinases is a promising pharmacological method for treating COVID-19 (PAKs). One of the most essential constituents of propolis, caffeine acid phenethyl ester (CAPE) has been shown to down regulate RAC (a signalling protein found in human cells), serving as a RAC/CDC42-activated kinase 1 (PAK1) blocker. These findings support the idea that CAPE could be used to prevent or slow Corona virus-



induced lung fibrosis (Maruta and He, 2020).

Although propolis is one of the safest natural remedies, it is possible that its constituents may cause side effects especially allergic reactions. As a result, when used to avoid or treat COVID-19 hypersensitivity reactions should be considered (Kurek-Gorecka *et al.*, 2020). Molecular docking revealed that Tetra gonula aff. biroi propolis compounds have the ability to inhibit SARS-CoV-2 protease activity. Sulabiroins-A had the highest binding affinity, followed by (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone and broussoflavonol F with binding affinity values of -8.1 kcal/mol, -7.9 kcal/mol and -7.9 kcal/mol respectively, with binding similarity of more than 50 per cent compared to N3 and SARS-CoV-2 main protease interaction. Since they interacted with His41 and Cys145 in the active site of SARS-CoV-2's main protease, these three compounds may be a promising drug candidate for COVID-19 (Liya *et al.*, 2021). According to Shaldam *et al.* (2020), p-coumaric acid, ellagic acid, kaemferol and quercetin are the most promising bioactive compounds derived from honey and propolis on 2019-nCoV active sites (RdRb and Mpro). These bioactive compounds were also discovered to have antiviral activity against the common cold human rhinovirus an RNA virus similar to SARS-CoV-2. Overall, we suggest additional *in vivo* investigations to determine the predicted affinity of the selected compounds against the novel Corona virus (COVID-19) target enzymes based on our theoretical studies and previous *in vitro* confirmatory studies.

Ali and Kunugi (2021) investigated the anti-COVID-19 effects of bee honey and propolis in the literature in order to improve the use of these convenient items as prophylactic or adjuvant drugs for people infected with extreme acute respiratory syndrome-Corona virus-2 (SARS-CoV-2). Flavonoids in propolis and honey (caffeic acid phenyl ester, rutin, naringin, luteolin and artepillin) have been shown in molecular simulations to inhibit viral spike fusion in host cells, viral-host interactions that cause the cytokine storm and viral replication. Rutin, propolis ethanolic extract and propolis liposomes inhibited non-structural proteins of SARS-CoV-2 *in vitro* and these compounds along with naringin inhibited SARS-CoV-2 infection in Vero E6 cells similar to the potent antiviral drug remdesivir. Propolis extracts delivered via nanocarriers have better antiviral effects than ethanolic extracts against SARS-CoV-2. In line with this COVID-19 patients who received green Brazilian propolis or a combination of honey and *Nigella sativa* had faster viral clearance, symptom recovery and hospital discharge as well as lower mortality, than those who received standard treatment alone. As a result, using bee products as an adjuvant treatment for COVID-19 may be helpful. Elwakil *et al.* (2021) found that the chemical composition of Egyptian propolis samples varied depending on the botanical sources and geographical distribution. The anti-COVID-19 activity of Menoufia propolis was found to be high. "Naggara *et al.* (2020) revealed that several *in vitro* and *in silico* studies confirmed anti-corona virus activity of propolis flavonoids like chrysin and kaempferol which were found to inhibit virus replication *in vitro* (Debiaggi *et al.*, 1990) and quercetin and its derivatives which inhibit the SARS-CoV-1 and MERS-CoV.". Apart from the protective effects of propolis phenolics and flavonoids against COVID-19 it could be used as adjuvant therapy to regulate the severe inflammatory reaction, particularly the cytokine storm associated with COVID-19 infection based on recent and previous findings about propolis immunomodulator and antiviral activity.

Cannabis sativa Pollen:

Cannabis sativa L. is one among the oldest plant species known in medicine and one that has been most studied with respect to its phytochemistry (Turner *et al.*, 1980). *Cannabis sativa* L., also called as Indian hemp is a herbaceous annual plant, cultivated mainly in Central Asia (India and China) since ancient times (Russo *et al.*, 2008). It's been used as a source of fibres, oil, food and medicine furthermore as for religious purposes over the centuries. It



contains a variety of chemically active substances like cannabinoids, terpenoids, flavonoids and alkaloids (Andre *et al.*, 2016). The foremost active compounds found in *Cannabis sativa* L. are the cannabinoids a class of terpenophenolic compounds accumulated within the trichome cavity of the female flowers (Brenneisen, 2007). Being wind pollinated staminate and dioecious hemp plants produce huge amounts of pollen those are attractive to bees but hemp does not produce any nectar the pollen rich nature of the flowers can make hemp an ecologically valuable crop (Briens and Arathi, 2019).

Ross *et al.* (2005) reported that mainly two glycosides were identified as kaempferol 3-O-sophoroside and quercetin 3-O-sophoroside by spectroscopic methods including high-field two-dimensional NMR experiments in pollen grain collected from male plants of *Cannabis sativa* L.

Rothschild *et al.* (2004) conducted an experiment and identified 68 compounds in the chemosphere of *Cannabis sativa* L. pollen in male and female plants of two cultivated varieties (Northern Lights and Hawaian Indica) by using coupled gas chromatography and mass spectrometry (GC-MS) methods. Twenty compounds were monoterpenes including the five major components: terpinolene, b-pinene, bmyrcene (E)-b-ocimene and limonene; twenty five were sesquiterpenes and other 23 were of mixed biogenetic origin, including benzylalcohol and 3-methyl-1-butanol which occurred only in pollen; two pyrazines occurred in Northern Lights females.





Chapter-5

POLLEN SOURCE

Monofloral and Polyfloral Bee Pollen:

Bee pollen is categorised as monofloral, bifloral or polyfloral depending on the number of floral species from which the bees collect pollen grains. The chemical composition of pollen as well as its nutritional and medicinal properties is determined by its composition (Denisow and Denisow-Pietrzyk, 2016).

Kostic *et al.* (2020) suggested that monofloral bee collected sunflower pollen has a number of attractive properties that could be used in food formulation. Lipids, proteins and carbohydrates are likely to be the most abundant macronutrients. Pollen has a high energetic value of 400 kcal/100 g due to these macronutrients. Pollen can also be used as a source of carotenoids an essential phytochemical according to Raman spectroscopy. The use of 0.1 per cent pollen suspension as an emulsifier in real-world systems has shown that it has excellent emulsifying qualities as measured by activity indices and emulsifying stability. The prepared pollen suspension has non-foaming characteristics. These non-foaming properties are needed in some food systems. In order to make any recommendations further research into different monofloral bee pollen samples is needed to determine specific emulsifying property ranges.

Cannabis is a dioecious species which means that the male and female flowers are produced on separate plants. There are several monoecious *cannabis* varieties with male and female flowers on the same plant and stress may also cause male flowers to appear on female plants but these are exceptions to the plant's dioecious existence. Male *Cannabis* plants bloom for two to four weeks and can yield 3,50,000 pollen grains in a single flower. Pollen is carried to female plants by the wind and when conditions are favourable it can fly great distances. Pollen is collected by bees but they are not attracted to female flowers and therefore do not contribute to pollination. Pollination reduced the yield of essential oils in *Cannabis* flowers by 56 per cent (Meier and Mediavilla, 1998). To make hemp grain which is used for food, feed and oil male plants and pollen are needed. Male hemp plants grow flowers that do not contain nectar but are extremely rich in pollen so fibre hemp does not require pollination. They can be grown without pesticides, herbicides or chemical fertilisers making them both safe for bees and a valuable source of nutritious pollen. Another great advantage of Hemp for bees is that it blooms in the fall after other mass flowering crops have finished blooming providing an excellent pollen source for bees and thereby improving their overall health.



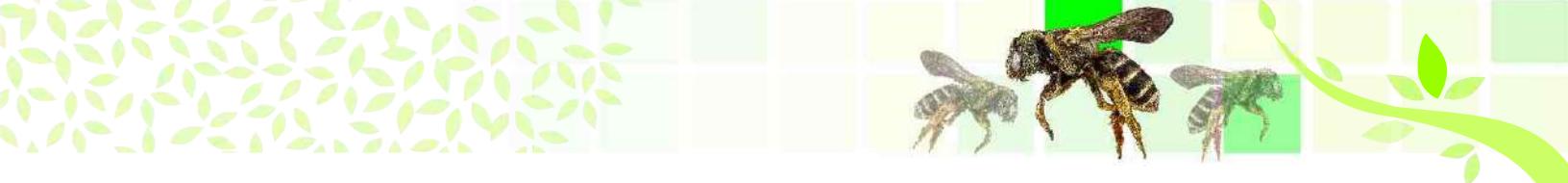
The researchers also noted that access to essential phytochemicals through pollen from a variety of plant sources is critical for honey bee survival and pathogen tolerance allowing them to live longer and healthier lives.

Taha *et al.* (2019) reported that amino acid composition and protein content of bee-pollens from major pollen floral sources in Al-Ahsa, Saudi Arabia in order to determine the nutritive value of pollen protein in relation to honeybee and adult human requirements. Bee pollens collected from date palm have high levels of amino acid concentrations and crude protein content while bee pollen collected from sunflowers have low level of crude protein and desirable amino acids. In bee-pollens collected from the five major floral sources a total of eighteen amino acids both essential and non-essential were found. Date palm has the highest concentrations of individual amino acids including cysteine, arginine, tyrosine, tryptophan and lysine while alfalfa bee pollen has the highest concentrations of leucine, isoleucine, phenylalanine, proline and valine. Rape bee pollen has the highest concentrations of aspartic acid summer squash has the highest concentrations of histidine. The basic amino acids leucine, isoleucine, methionine, valine and phenylalanine were found in the lowest concentrations in sunflower bee pollen. The essential amino acids in bee pollen from date palm, alfalfa, summer squash and rape seed surpassed the requirements of honey bee regardless of arginine and methionine. Methionine was the limiting amino acid among the five amino acids tested in bee pollen. The concentrations of important amino acids in the pollen grains tested were variable and significantly associated with the botanical origin of the pollen grains. Pollen from alfalfa, date palms and summer squash has been discovered to be a rich source of protein and amino acids for both bees and humans.

Spulber *et al.* (2018) reported that physicochemical properties of Romanian fresh bee pollen samples show a great variation among plant sources. *Prunus* L. sp., *Aesculus* sp. and *Brassica* sp. pollen are important sources of protein, *Plantago lanceolata* contains the greatest macronutrient (carbohydrates) and the highest total lipids content was found in *Brassica* sp. pollen. The important source of mineral elements content of fresh bee pollen gives a high nutritive value of the product important in human food.

Table 2: Pollen Producing Plants (Sallibartan, 2016)

Family	Scientific Name	Family	Scientific Name	Family	Scientific Name
Aceraceae	<i>Acer campestre</i>	Cornaceae	<i>Cornus sanguinea</i>	Lamiaceae	<i>Thymus vulgaris</i>
Apiaceae	<i>Anthriscus vulgaris</i>	Cornaceae	<i>Cornus mas</i>	Liliaceae	<i>Asparagus acutifolius</i>
Actinidiaceae	<i>Actinidia deliciosa</i>	Cupressaceae	<i>Cupressus arizonica</i>	Liliaceae	<i>Allium sativum</i>
Apiaceae	<i>Heracleum sphondylium</i>	Cucurbitaceae	<i>Cucurbita pepo L</i>	Liliaceae	<i>Lilium sp.</i>
Apiaceae	<i>Ferula communis</i>	Cucurbitaceae	<i>Bryonia dioica</i>	Liliaceae	<i>Allium cepa</i>
Araliaceae	<i>Hedera helix</i>	Cupressaceae	<i>Thuja occidentalis</i>	Lamiaceae	<i>Thymus serpyllum</i>
Apiaceae	<i>Chaerophyllum sp.</i>	Ericaceae	<i>Erica arborea</i>	Liliaceae	<i>Asphodelus fistulosus</i>
Apiaceae	<i>Daucus carota</i>	Euphorbiaceae	<i>Mercurialis annua</i>	Lythraceae	<i>Lythrum salicaria</i>
Asteraceae	<i>Centaurea solstitialis</i>	Ericaceae	<i>Calluna vulgaris</i>	Myrtaceae	<i>Eucalyptus globulus</i>
Apocynaceae	<i>Vinca minor L</i>	Ericaceae	<i>Arbutus unedo</i>	Malvaceae	<i>Malva sylvestris</i>



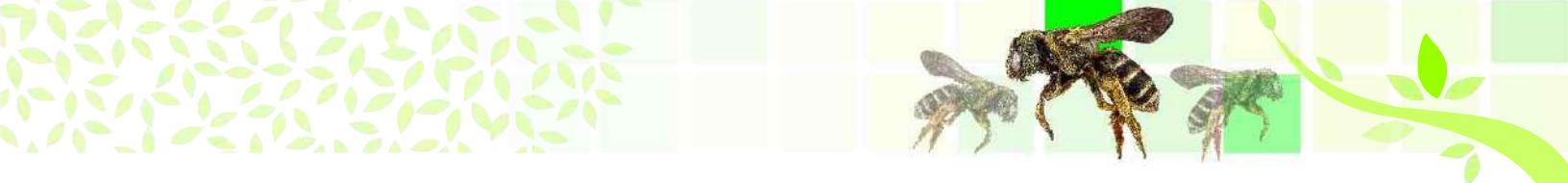
Asteraceae	<i>Chondrilla juncea L</i>	Ericaceae	<i>Vaccinium</i>	Oleaceae	<i>Ligustrum ovalifolium</i>
Asteraceae	<i>Arctium sp.</i>	Fabaceae	<i>Robinia pseudoacacia</i>	Onagraceae	<i>Epilobium angustifolium</i>
Asteraceae	<i>Hypochoeris radicata</i>	Fabaceae	<i>Medicago sativa</i>	Oleaceae	<i>Fraxinus excelsior</i>
Asteraceae	<i>Carduus sp.</i>	Fabaceae	<i>Lupinus angustifolius</i>	Primulaceae	<i>Dodecatheon clevelandii</i>
Asteraceae	<i>Cirsium sp.</i>	Fabaceae	<i>Dorycnium pentaphyllum</i>	Pinaceae	<i>Pinus sylvestris</i>
Asteraceae	<i>Leucanthemum vulgare</i>	Fabaceae	<i>Vicia faba</i>	Plantagi naceae	<i>Plantago sp.</i>
Asteraceae	<i>Achillea millefolium</i>	Fabaceae	<i>Ulex europaeus</i>	Polygonaceae	<i>Rumex acetosa</i>
Asteraceae	<i>Eupatorium cannabinum</i>	Fabaceae	<i>Cytisus scoparius</i>	Polygonaceae	<i>Fagopyrum esculentum</i>
Asteraceae	<i>Centaurea jacea</i>	Fabaceae	<i>Galega officinalis</i>	Papaveraceae	<i>Papaver rhoeas</i>
Asteraceae	<i>Centaurea cyanus</i>	Fabaceae	<i>Melilotus sp.</i>	Poaceae	<i>Zea mais</i>
Asteraceae	<i>Artemisia sp.</i>	Fabaceae	<i>Lotus uliginosus</i>	Rosaceae	<i>Fragaria</i>
Asteraceae	<i>Taraxacum officinale</i>	Fabaceae	<i>Lotus corniculatus</i>	Ranuncu laceae	<i>Helleborus niger</i>
Asteraceae	<i>Helianthus annuus</i>	Fabaceae	<i>Phaseolus vulgaris L.</i>	Rhamnaceae	<i>Rhamnus alaternus</i>
Betulaceae	<i>Betula pendula</i>	Fabaceae	<i>Onobrychis viciifolia</i>	Rosaceae	<i>Rubus idaeus</i>
Betulaceae	<i>Corylus avellana</i>	Fabaceae	<i>Pisum sativum L</i>	Rhamnaceae	<i>Frangula alnus</i>
Betulaceae	<i>Alnus glutinosa</i>	Fabaceae	<i>Castanea sativa</i>	Rosaceae	<i>Prunus armeniaca</i>
Bignoniaceae	<i>Catalpa speciosa</i>	Fabaceae	<i>Sophora japonicum</i>	Rosaceae	<i>Prunus dulcis</i>
Bignoniaceae	<i>Campsis radicans</i>	Fabaceae	<i>Trifolium pratense</i>	Rosaceae	<i>Crataegus sp.</i>
Bignoniaceae	<i>Sympytum officinalis</i>	Fabaceae	<i>Glycine max</i>	Rosaceae	<i>Pyrus communis</i>
Brassicaceae	<i>Rapistrum rugosum</i>	Fabaceae	<i>Vicia vilosa</i>	Rosaceae	<i>Prunus sp.</i>
Bignoniaceae	<i>Echium vulgare</i>	Fagaceae	<i>Fagus sylvatica</i>	Rosaceae	<i>Pyrus sp.</i>
Brassicaceae	<i>Brassica napus</i>	Fabaceae	<i>Trifolium sp.</i>	Rosaceae	<i>Prunus avium</i>
Brassicaceae	<i>Sisymbrium officinale</i>	Fagaceae	<i>Quercus sp.</i>	Rosaceae	<i>Prunus persica</i>
Brassicaceae	<i>Sinapis alba</i>	Grossulariceae	<i>Ribes rubrum</i>	Rosaceae	<i>Sorbus aucuparia</i>
Brassicaceae	<i>Brassica rapa</i>	Hydrangeaceae	<i>Philadelphus coronarius</i>	Rosaceae	<i>Filipendula ulmaria</i>



Brassicaceae	<i>Raphanus sativus</i>	Hippocastaneae	<i>Aesculus hippocastanum</i>	Rosaceae	<i>Rosa sp.</i>
Buxaceae	<i>Buxus sempervirens</i>	Hypericineae	<i>Hypericum sp.</i>	Rosaceae	<i>Rubus fructicosus</i>
Caprifoliacee	<i>Viurnum tinus</i>	Hydrophy laceae	<i>Phacelia tanacetifolia</i>	Rutaceae	<i>Citrus clementina</i>
Caprifoliaceae	<i>Sambucus nigra</i>	Lamiaceae	<i>Prunella vulgaris</i>	Simaroubaceae	<i>Ailanthus altissima</i>
Caprifoliaceae	<i>Silene sp.</i>	Lamiaceae	<i>Hyssopus officinalis</i>	Solanaceae	<i>Solanum lycopersicum</i>
Campanulaceae	<i>Jasione sp.</i>	Lamiaceae	<i>Rosmarinus officinalis</i>	Salicaceae	<i>Salix caprea</i>
Cistaceae	<i>Helianthemum</i>	Lamiaceae	<i>Lavendula sp</i>	Salicaceae	<i>Populus sp.</i>
Cistaceae	<i>Cistus sp.</i>	Lamiaceae	<i>Salvia pratensis</i>	Tiliaceae	<i>Tilia x vulgaris</i>

Table 3: Details of Bee Flora a Source of Pollen and Nectar (As per Indian Conditions)

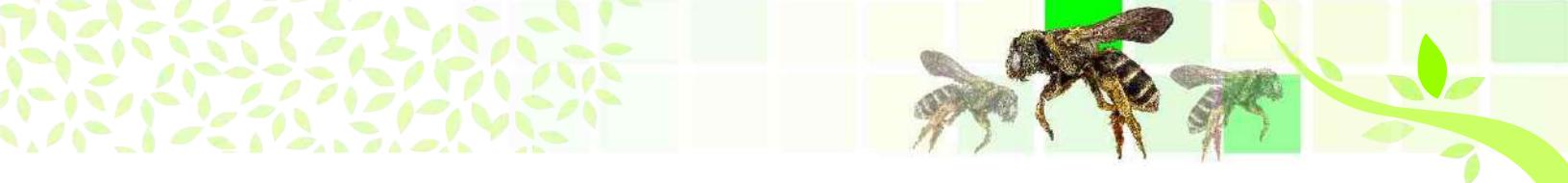
S.No.	Local Name	Scientific Name	Flowering Period	Source	Remark
1	Mustard	<i>Brassica sp.</i>	January-February	Pollen and Nectar	Rich in Selenium having high inflammatory effects and high source of Magnesium
2	Pigeon Pea	<i>Cajanus cajan</i>	September-November	Nectar	Leaves are used to staunch blood as an analgesic, cure gingivitis, stomatitis
3	Sesame	<i>Sesamum indicum</i>	September	Nectar	Source of proteins, Vitamin B, antioxidants, help in blood control and lower cholesterol
4	Litchi	<i>Litchi chinensis</i>	March-April	Nectar	Treatment of cough, flatulence, diabetes, neuralgic pain, hernia like conditions
5	Apple	<i>Malus domestica</i>	February-April	Pollen and Nectar	Lower risk of diabetes, prevent cancer, fight asthma



7	Peach	<i>Prunus persica</i>	February-April	Pollen and Nectar	Packed with nutrients and antioxidants, rich in vitamins, minerals
8	Coconut	<i>Cocos nucifera</i>	May-June	Nectar	Cure diabetes, high cholesterol
9	Bottle brush	<i>Callistemon lanceolatus</i>	March-April	Nectar	Have antibacterial, antifungal, antioxidant, pharmaceutical properties
10	Drumstick	<i>Moringa oleifera</i>	February	Nectar	Reduction in blood sugar and cholesterol, have antioxidant and anti inflammatory effects
11	Semal (Cotton Tree)	<i>Bombax ceiba</i>	January-February	Nectar	Cure gastrointestinal, skin, urogenital diseases, also have anti inflammatory, anti oxidant properties
12	Bakas	<i>Justicia gendarussa</i>	January-February	Nectar	Cure bronchitis, inflammations, dyspepsia, eye diseases
13	Jamun	<i>Syzygium cumini</i>	April	Nectar	Mixture of bio components like tannins and carbohydrates, astringent to fight dysentery
14	Mahua	<i>Madhuca latifolia</i>	April	Nectar	Cure cough, bronchitis, piles, impotence and general debility
15	Karanj	<i>Pongamia glabra</i>	April-May	Nectar	All parts are used in managing constipation and also has a laxative property
16	Berseem	<i>Trifolium alexandrinum</i>	April-May	Nectar	Used as a cover crop suppressing weeds providing nitrogen
17	Lemon	<i>Citrus limon</i>	February-March	Pollen and Nectar	Good source of Vitamin C, controls weight, prevent kidney stones, cancer risk
18	Sunflower	<i>Helianthus annus</i>	November-December	Nectar	Used for pulmonary infections remedy, substitute of quinine so used in the treatment of malaria



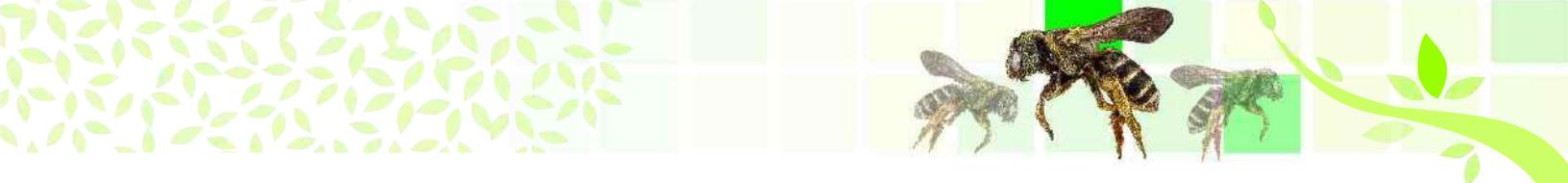
19	Maulsari (Spanish cherry)	<i>Mimusops elengi</i>	April-May	Nectar	Have antipyretic, anti inflammatory and anti hyperlipidemic properties
20	Toon (Chinese Mahogany)	<i>Cedrela toona</i>	March-April	Nectar	Improve strength and immunity, cures cold
21	Eucalyptus	<i>Eucalyptus globulus</i>	November-April	Nectar	Cure arthritis, ulcers, diabetes, fever, bladder diseases
22	Sheesham (Indian Rosewood)	<i>Dulbergia sissoo</i>	April-May	Nectar	Have abortifacient, anthelmintic, antipyretic, aphrodisiac properties
23	Maize	<i>Zea mays</i>	May-June	Pollen	Its silk used for bladder infection, diabetes, blood pressure, fatigue treatments
24	Coriander	<i>Coriandrum sativum</i>	January-February	Pollen and Nectar	Cures mouth ulcers, strengthen bones, good for vision, remedy for conjunctivitis, regulates diabetes
25	Tobacco	<i>Nicotiana tabaccum</i>	January-February	Pollen and Nectar	Act as a respiratory stimulant as bronchial asthma, have diuretic, antispasmodic properties
26	Fennel	<i>Foeniculum vulgare</i>	January-February	Pollen and Nectar	Used for indigestion, suppress appetite, have cancer fighting properties
27	Khesari (Indian Pea)	<i>Lathyrus sativus</i>	February	Pollen and Nectar	Only known dietary source for L-homoarginine
28	Date palm	<i>Phoenix dactylifera</i>	February-March	Pollen	Have anti inflammatory properties, reduce risk of cancer and diabetes
29	Safflower	<i>Carthamus tinctorius</i>	April-May	Pollen and Nectar	Cure rheumatism and paralysis, mouth ulcers
30	Mirchaiya	<i>Croton sparsiflorus</i>	March-May	Pollen	Have antibacterial properties and also used to cure malaria
31	Bhang	<i>Cannabis sativa</i>	April-May	Pollen	Have psychoactive effects, prevent nausea and vomiting and muscle spasms also



32	Banana	<i>Musa sp.</i>	April-July	Nectar	Moderate blood sugar levels, digestive health, improve insulin sensitivity
33	Neem	<i>Azadirachta indica</i>	April	Pollen and Nectar	Used for leprosy, eye disorders, skin ulcers, diabetes and liver problems
34	Aonla	<i>Emblica officinalis</i>	April	Pollen	Have acrid, cooling, diuretic and laxative properties
35	Guava	<i>Psidium guajava</i>	April	Pollen and Nectar	Lower blood sugar, boost heart health, have anticancer effect
36	Mexican creeper	<i>Antigonon leptopus</i>	July-November	Pollen and Nectar	Possess antimicrobial, antioxidant, hepatoprotective, analgesic, anti inflammatory activities
37	Watermelon	<i>Citrullus lanatus</i>	March-May	Pollen and Nectar	Lower inflammation and oxidative stress, prevent macular degeneration and hydrate the body
38	Muskmelon	<i>Cucumis melo</i>	March-May	Pollen and Nectar	Abundant in Vitamin C that boosts immunity, equips our system with new WBCs
39	Palmyra palm	<i>Borassus flabellifer</i>	March-June	Nectar	Have analgesic and anti pyretic effects, anti inflammatory, haematological and immunosuppressant properties
40	Tamarind	<i>Tamarind indica</i>	April-June	Pollen and Nectar	Cure constipation, liver and gallbladder problems and stomach disorders



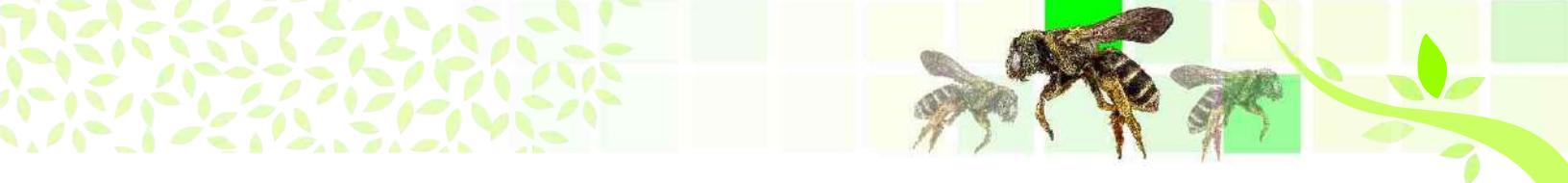
41	Crape myrtle	<i>Lagerstroemia indica</i>	June	Nectar	Cure diabetes, regulates blood pressure, fights obesity
42	Cotton	<i>Gossypium hirsutum</i>	December-January	Nectar	Used for nausea, dysentery, nerve pain
43	Jowar	<i>Sorghum vulgare</i>	February-March	Nectar	Improves digestion, blood sugar level, good source of fibre
44	Patsan	<i>Hibiscus cannabinus</i>	August-September	Pollen	Used as a poultice on pain, cure dysentery
45	Buckwheat	<i>Fagopyrum esculentum</i>	Late summer to early Autumn	Pollen and Nectar	Improve blood flow, treat diabetes and prevent atherosclerosis
46	Carrot	<i>Daucus carota</i>	March-April	Pollen and Nectar	Source of Beta carotene which act as an antioxidant
47	Onion	<i>Allium cepa</i>	May-June	Pollen and Nectar	Manages Diabetes, prevent cancer risk
48	Raddish	<i>Raphanus sativus</i>	December-March	Pollen and Nectar	Used for high cholesterol, bronchitis, cough, stomach and intestinal disorders
49	Garlic	<i>Allium rubellum</i>	September-October	Pollen and Nectar	Cure high blood pressure, high cholesterol and hardening of arteries
50	Asparagus	<i>Asparagus officinalis</i>	December-January	Pollen and Nectar	Source of Vitamins A,C and K, improves digestion and lower Blood pressure
51	Cherry	<i>Prunus avium</i>	March-April	Nectar	Rich in antioxidants and anti inflammatory compounds, improve arthritis



52	Strawberry	<i>Fragaria sp.</i>	February-March	Nectar	Lower Blood pressure and guard against cancer, packed with vitamin fiber and also are a sodium free
53	Cucumber	<i>Cucumis sativus</i>	July-August	Nectar	Reduce bad breath, protect bones and support heart health
54	Walnut	<i>Juglans regia</i>	March-April	Pollen	Rich in antioxidants, rich source of Omega-3s decrease inflammation, lower blood pressure
55	Amaltas	<i>Cassia fistula</i>	May-June	Nectar	Used in joint pain, migraine, chest pain and blood dysentery
56	Pomegranate	<i>Punica granatum</i>	March-April	Nectar	Rich in antioxidants that helps in removal of free radicals, protect cells from damage
57	Lyonia	<i>Pieris ovalifolia</i>	April-May	Nectar	Used externally as an infusion to treat skin diseases
58	Babul	<i>Accacia arabica</i>	February-March	Nectar	Used as datum for cleaning teeth, reduce plaque and inflammation
59	Indian butter Tree	<i>Bassia butyracea</i>	March	Pollen	Have antioxidant, anti inflammatory properties
60	Oak	<i>Quercus incana</i>	March	Nectar	Used as an antiseptic
61	Datura	<i>Datura fastuosa</i>	Annual	Pollen	Its seeds are analgesic, anthelmintic and anti inflammatory
62	Hisalu	<i>Rubus ellipticus</i>	March-April	Nectar	Treat indigestion and headaches



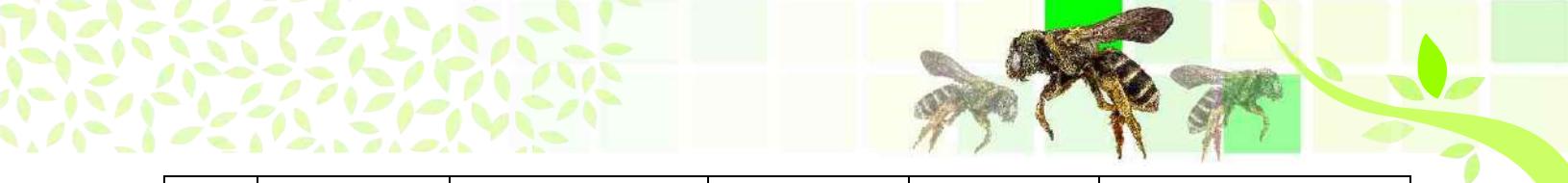
63	Kachnar	<i>Bauhinia variegata</i>	March-November	Nectar	Treat Hemorrhoids, hypothyroidism, have anti cancer properties, cure diarrhea
64	Japanese Persimmon	<i>Diospyros kaki</i>	April	Nectar	Treat cough, hypertension, paralysis, frostbite, burns and bleedings
65	Kalthunia	<i>Plectranthus sp.</i>	September-November	Nectar	Possess antimicrobial, antifungal, anti inflammatory, anti diabetic, anti malarial properties
66	Orange Jasmine	<i>Murraya exotica</i>	June-July	Nectar	Ground bark of stem used as antidote in snake bites while ground roots cure body ache
67	Bitter Gourd	<i>Mimordica charantia</i>	July-August	Nectar	Treat asthma, constipation, diabetes, leprosy, malaria, ulcer
68	Apricot	<i>Prunus armeniaca</i>	February-March	Nectar	High in antioxidants, promote eye health, high in potassium, promote gut health
69	Indian barberry	<i>Berberis aristata</i>	February-March	Nectar	Have antibacterial, antiperiodic, antidiarrheal, anticancer properties
70	Musk rose	<i>Rosa moschata</i>	April-May	Pollen	Treat abdominal spasm and diarrhoea
71	Indian Cherry/Glue berry	<i>Cordia dichotoma</i>	March-April	Pollen	Treat dyspepsia, fever, diarrhoea, leprosy, gonorrhoea, ulcer



72	Loquat	<i>Eriobotrya japonica</i>	September - January	Nectar	Treat cough, chronic bronchitis, inflammation, diabetes
73	Finger Millet	<i>Eleusine coracana</i>	August-September	Pollen	Reduce anxiety
74	Salix (Weeping willow)	<i>Salix babylonica</i>	February-March	Pollen	Treat mastitis, toothaches and scalds
75	Mehal (Wild Himalayan Pear)	<i>Pyrus pashia</i>	March-April	Nectar	Treat conjunctivitis, have laxative and astringent properties
76	Mulberry	<i>Morus indica</i>	March	Pollen	Treat diabetes, high cholesterol levels, constipation, hair loss, premature graying
77	Padam (Wild Berry)	<i>Prunus cerasoides</i>	November-December	Nectar	Treat nausea, vomiting and gastritis
78	Chestnut	<i>Esculentus hippocastanum</i>	May-June	Nectar	Treat severe cough, colds, bronchitis and diarrhoea
79	Poppy	<i>Papaver somniferum</i>	March-April	Pollen	Relax smooth muscle tone, treat diarrhoea
80	Sisal	<i>Agave cantala</i>	July-August	Pollen	Lower blood pressure
81	Wild Rose	<i>Rosa macrophylla</i>	April-May	Pollen	Good source of essential fatty acids, reduce cancer
82	Kuntze	<i>Centratherum anthelminticum</i>	October-November	Nectar	Treat fever, cough and diarrhoea
83	Lodh	<i>Symplocos beddomei</i>	November	Pollen	Used as a remedy for uterine complaints, vaginal diseases
84	Holigarna	<i>Holigarna grahamii</i>	December-January	Pollen and Nectar	Treat inflammation, obesity, tumor, cancer and skin diseases



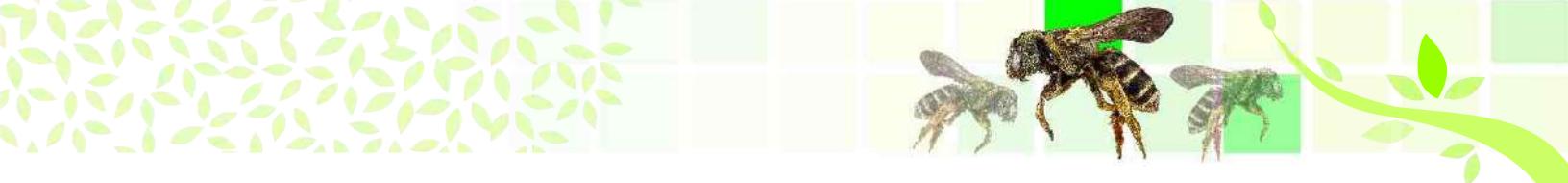
85	Water Star	<i>Dysophylla stellata</i>	December-January	Pollen and Nectar	Treat respiratory infections, nausea, constipation and other digestive issues
86	Tilia	<i>Tilia cordata</i>	May - July	Pollen and Nectar	Promote relaxation, have diuretic effects, lower blood pressure
87	Pride of India	<i>Lagerstroemia parviflora</i>	May	Pollen	Treat urinary tracts, blood pressure, type II diabetes mellitus
88	Prishnaparni	<i>Uraria picta</i>	July-September	Nectar	Have antiseptic
89	Arjun	<i>Terminalia arjuna</i>	May-June	Pollen	Have antioxidant, anti inflammatory and antimicrobial properties
90	Golden rod	<i>Solidago canadensis</i>	July-October	Pollen	Reduce pain and swelling, also used for rheumatism, eczema
91	Rangoon creeper	<i>Combretum indicum</i>	September-October	Pollen	Treat diarrhoea, rheumatism, cough, headaches
92	Moss rose	<i>Portulaca grandiflora</i>	May-August	Pollen	Treat hepatitis, cirrhosis of liver with ascites, swelling and pain in pharynx
93	New York aster	<i>Aster novibelgii</i>	September-November	Pollen and Nectar	Treat venereal diseases
94	Himalayan balsam	<i>Impatiens glandulifera</i>	July-September	Pollen and Nectar	Used in homeopathy to treat anxiety
95	Saffron	<i>Crocus sativus</i>	October-November	Pollen and Nectar	Used for asthma, cough, loosen phlegm
96	Sapota	<i>Achras zapota</i>	October-November	Pollen and Nectar	Promotes gut health, stronger bones, prevents cancer



97	Papaya	<i>Carica papaya</i>	May-August	Pollen and Nectar	Treat gastrointestinal tract disorders, intestinal parasites infections
98	Ber	<i>Ziziphus mauritiana</i>	July-October	Pollen and Nectar	Improve blood circulation, good for bones
99	Plum	<i>Prunus domestica</i>	February-March	Pollen and Nectar	Lower inflammation, constipation relief, rich in antioxidants
100	Cauliflower	<i>Brassica oleracea var. botrytis</i>	April-May	Pollen and Nectar	Keeps bones healthy, reduce blood pressure, strengthen immune system
101	Cabbage	<i>Brassica oleracea var. capitata</i>	April-May	Pollen and Nectar	Rich in vitamin C and K lower blood pressure, cholesterol
102	Okra	<i>Abelmoschus esculentus</i>	June-September	Pollen and Nectar	Rich in nutrients, antioxidants, lower heart disease, blood sugar
103	Turnip	<i>Brassica rapa</i>	February-March	Pollen and Nectar	Cancer prevention properties, rich in antioxidant lutein thus improves eye health
104	Chilli	<i>Capsicum annum</i>	July-September	Pollen and Nectar	Improves digestion health and metabolism, supports cardiovascular health
105	Fenugreek	<i>Trigonella foenumgraecum</i>	March-April	Pollen and Nectar	Used for diabetes, polycystic ovary syndrome and obesity
106	Rice	<i>Oryza sativa</i>	May-September	Pollen	Essential source of Vitamin B1, helps in good bowel movement, stabilize blood sugar levels



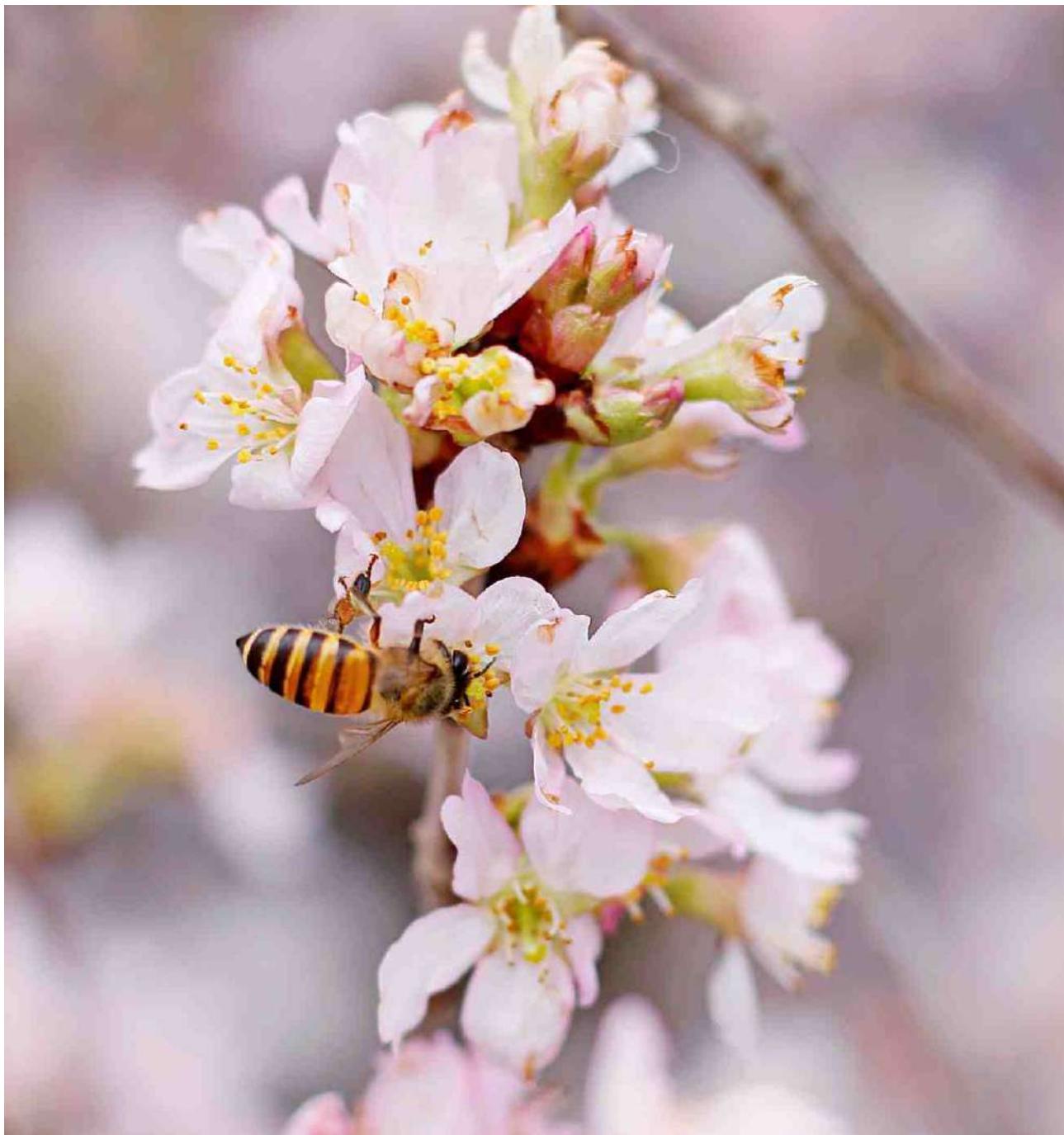
107	Chickpea	<i>Cicer arietinum</i>	February-March	Pollen and Nectar	Rich source of Vitamins, fiber, minerals, protein improve digestion
108	Rubber	<i>Hevea brasiliensis</i>	March	Nectar	Used for healing wounds, recommend in decoction
109	Pink Balsam	<i>Impatiens balsamina</i>	July-August	Nectar	Treat Fractures, rheumatism and fingernails
110	Hibiscus	<i>Hibiscus rosasinensis</i>	February-April	Pollen and Nectar	Treat cystitis, venereal diseases, feverish illnesses, bronchial catarrh
111	Gulmohar	<i>Delonix regia</i>	May-June	Pollen and Nectar	Have anti diabetic activity, antibacterial activity, anti microbial activity
112	Portulaca	<i>Portulaca oleracea</i>	May-September	Pollen	Treat burns, headache, arthritis
113	Cosmos	<i>Cosmos bipinnatus</i>	September-October	Pollen and Nectar	Have antioxidant potential, anti diabetic, anti hypersensitive, bone protective effect
114	Calendula	<i>Calendula officinalis</i>	February-May	Pollen and Nectar	Prevent muscle spasms, reduce fever, treat cancer, stomach ulcers
115	Cyrus	<i>Cereus hexagonus</i>	March-April	Nectar	Control high blood pressure and heart diseases
116	Curry Patta	<i>Murraya koenigii</i>	March-April	Pollen and Nectar	Treat dysentery, constipation, eliminates bacteria, good for eyesight



117	Reetha	<i>Sapindus mukorossi</i>	April-May	Pollen and Nectar	Promote hair health, skin health, respiratory health, anti inflammatory effects
118	Brinjal	<i>Solanum melongena</i>	Mar-June, Sept-Dec	Pollen	Great source of vitamins and minerals, helps with digestion, heart health, anaemia
119	Kate math	<i>Amaranthus sp.</i>	September - October	Pollen	Treat fever, pain, asthma, diabetes, dysentery
120	Garden Pea	<i>Pisum sativum</i>	September - December	Pollen and Nectar	Its seed is fungistatic and spermicidal
121	Bottle gourd	<i>Lagenaria siceraria</i>	August - September	Pollen and Nectar	Reduce stress, benefit heart, skin, digestion
122	Sponge Gourd	<i>Luffa acutangula</i>	July-October	Pollen and Nectar	Rich in Vitamin A, C, iron, Magnesium and Vitamin B6, enhance vision, protect liver
123	Cluster bean	<i>Cyamopsis tetragonolobus</i>	June-August	Nectar	Lower the cholesterol level, help in digestion
124	Mango	<i>Mangifera indica</i>	January-April	Pollen	Rich in polyphenols and terpenoids, treat skin, cancer, stomach ulcers
125	Jackfruit	<i>Artocarpus heterophyllus</i>	Feb-March	Nectar	Control cholesterol, blood pressure, cancer, blood sugar, digestion
126	Tulsi	<i>Ocimum sanctum</i>	July-Sept/Mar-April	Pollen and Nectar	Contains Vitamin C, Anti-ageing properties, treat fever, kidney stones, eye health
127	Rose	<i>Rosa indica</i>	March-September	Pollen	Helps in digestion, improve bile secretion



128	Marigold	<i>Tagetes erecta</i>	September-December	Nectar	Heals skin wounds, burns, treat cancer, bursitis
129	Palas/Flame of Forest	<i>Butea monosperma</i>	February-April	Pollen and Nectar	Have anthelmintic properties, astringent property





Chapter-6

BENEFITS OF POLLEN FOR HONEY BEE HEALTH

Diet Requirements according to the Age of the Honey Bee and Importance of Pollen:

“A honeybee diet requirement changes with age resulting in an adapted physiology” (Zerck, 2013). Honey bees consume royal jelly, pollen, nectar and honey during their life cycle. The larvae are initially fed royal jelly with some pollen grains and after three days the cast of honey bees decides the form of feed because it affects the development of the genital organ. Worker bees are fed a diet that includes sugar, bee bread, pollen and water while queen bees are fed royal jelly. “Pollen is primarily eaten by bee nurses to feed at least three day old larvae.” (Di Pasquale *et al.*, 2013; Jean-Prost, 2005). Pollen is consumed by bees on their own from 42-72 hours of age to 8-9 days of age, when they hit their peak consumption rate. A larva needs 100 mg of pollen per day on average (Haydak, 1935). It causes gland production and pollen consumption decreases after 20 days necessitating the use of honey as a replacement for feed. Pollen is only needed in small amounts by old bees (Jean-Prost, 2005). As a result brood production is directly related to pollen consumption and colony organisation.

Pollen is needed by honey bees throughout the year. From April to November honey bees forage and collect pollen and during the rest of the year stored pollen is used as a source of food. Throughout the foraging season pollen quantity and quality are extremely significant (Requier *et al.*, 2015). Pollen with a specific composition is needed in the diet of worker honey bees. To meet the minimum dietary requirements pollen must contain ten specific amino acids (Cook *et al.*, 2003) as well as a high content of vitamins especially water-soluble vitamin B complex. However, it is still unknown whether fat soluble vitamins like vitamin A, vitamin D, vitamin E and vitamin K are needed in *Apis mellifera* diet (Herbert *et al.*, 1978). Pollen is made up of two types of metabolites: primary metabolites (carbohydrates, proteins, lipids, mineral matter, vitamins and water) and secondary metabolites (hormones, saponines, brassinosteroids, alkaloids, microorganisms, phenolic compounds and terpenic compounds). The majority of the primary metabolites are necessary components of the honey bee's diet and have a direct effect on normal development, growth and reproduction.

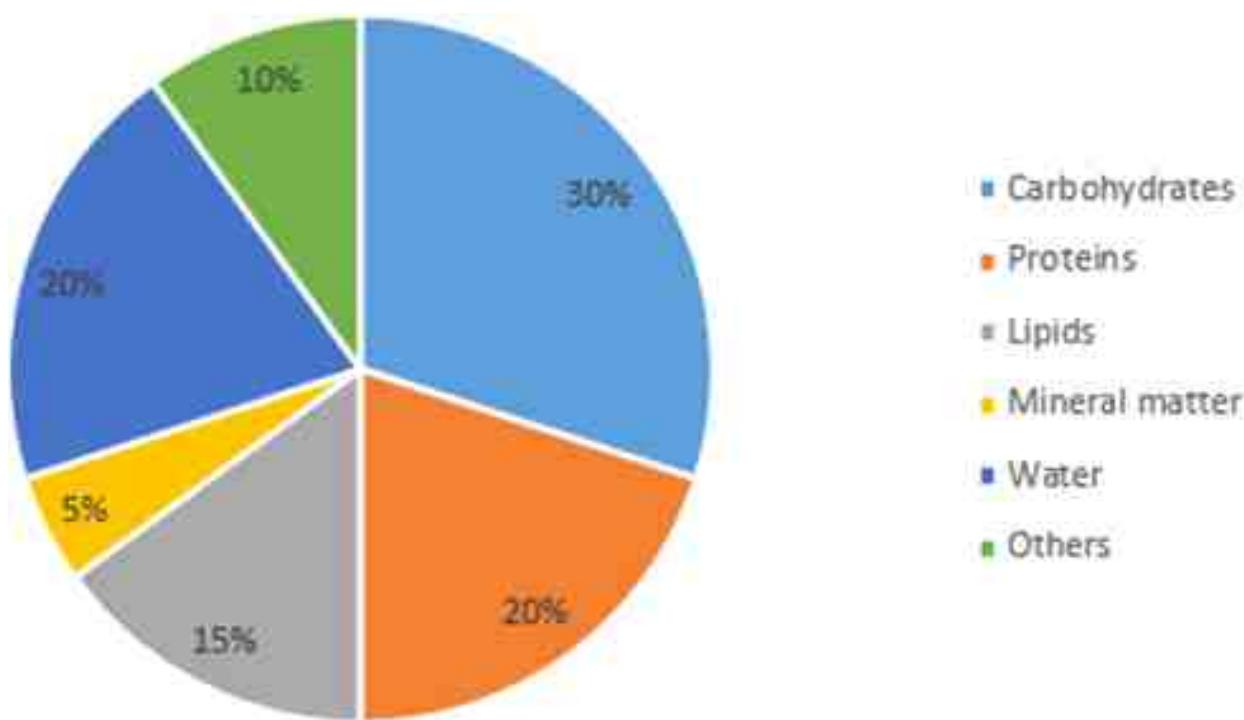


Figure 5: Composition of Pollens Harvested by Honey Bee (Todd and Bretherick, 1942; Jeanne, 1993)

Impact of Pollen Compounds on Bee Health and Resistance to Pesticides and Diseases:

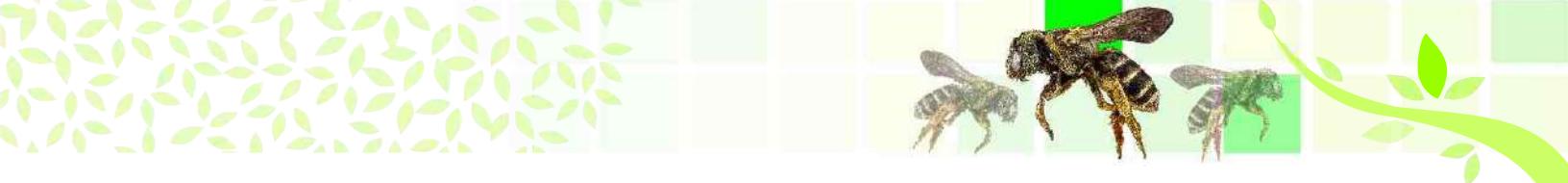
Pollen quality and functions are determined by its conservation state and collection. Hand harvested pollen different stages of fermentation and finally bee collected pollen, bee-bread can all be distinguished. Pollen that has been stored for five years will promote gland development in young bees while also increasing the life span of the bees and making egg production more efficient (Pain *et al.*, 1966). Pollen quality is measured by the age and composition of the pollen grains.

Pollen Quantity:

The amount of pollen collected is difficult to estimate because it is dependent on a number of factors including the size of the swarm, the size of the bees, colony behaviour, landscape resources and bloom time (Guerriat, 1996). Furthermore, the number of births varies greatly depending on the feed source. The more food that is brought into the hive the more bees that are produced. As a result, the emphasis is placed on colony building rather than nutritive value. The weight of pollen is more significant than the volume of pollen for this reason.

Pollen Quality:

Researchers came to various conclusions about the impact of high quality pollen on honey bee preferences. Pollen quality is influenced by honey bee foraging preferences (Cook *et al.*, 2003). Aside from flower structure, colour, sugar content and protein content some pollens are more appealing than others (Levin and Bohart, 1955). Sugar is not



particularly appealing since, the most desirable pollen obtained from *Brassica nigra* contains the least amount of sugar. Freshness, moisture content, colour and reflectivity are all factors that affect pollen attractiveness (Levin and Bohart, 1955). Proteins are needed in the diet of honey bees and a lack of proteins has been shown to have a major impact on the ability of individual honey bees to resist disease. At least ten basic amino acids should be present in sufficient quantities in beneficial pollen. The amount of essential amino acids in pollen can have an effect on nutritional quality and thus on honey bee growth and development (Cook *et al.*, 2003).

The Influence of Pollen Quality on Nosema Tolerance:

A certain level of tolerance to *Nosema ceranae* (a microfungus that infects honey bees and causes mass colony death) has been observed and it appears to be dependent on pollen quality. This quality is determined by comparing many monofloral pollens rather than pollen diversity (Di Pasquale *et al.*, 2013). Pollen diversity confers resistance to nosema in honey bees but it does not protect them from infection with *Nosema ceranae*.

Pesticides Resistance:

Pesticides have a deadly effect on honey bee survival. Neo-nicotinoid a form of insecticide molecule is particularly lethal for pollinators depending on dosage, body weight and other factors (Henry *et al.*, 2012). Young bees are rarely exposed to pesticides with the exception of chemicals spread in the hive to combat Varroa mites. Neonicotinoid molecules are carried to the hive by foraging bees and exposure occurs by trophallaxis. Pesticide resistance is higher in honey bees that are fed enough high quality pollen (Wahl and Ulm, 1983).

Beekeepers Feed Management:

“At the start of the season in April and at the end of the foraging season in September there is a lack of resources” (Mandel, 2015; Godet and Savoie, 2011). The availability of resources during the foraging season is critical for establishing winter food reserves. Honey bee colony growth and resistance are influenced by the floral environment to some degree (Godet and Savoie, 2011). Qualitative pollens rich in carbohydrates, proteins, lipids and minerals have two peaks *i.e.* in May-June and mid-July to mid-August (Di Pasquale *et al.*, 2013). Honey must be harvested in a regulated manner to ensure that the colonies feed requirements are met during the winter season. Beekeepers are mindful of the scarcity of food in the hives for colony survival as the winter season begins (Jean-Prost, 2005). Beekeepers nowadays feed honey bees various sources such as sugar, honey, syrup and candy at various times of the year (especially in the winter). In light of winter feed scarcity and global pollinator decline beekeepers should carefully observe the landscape to locate their hives in a lush floral setting as well as change their apiary practices during times of scarcity.

The pollen collection system entails installing a pollen grid trap with large holes enough to enable bees to reach the hive but small enough to prevent pollen balls from falling into the trap, resulting in pollen balls falling and being collected in the trap (Figure 6). Honey bee legs are adapted to the workers activities with the last pair of legs being bent to gather pollen and form a pollen ball for transport. The brush and comb on the second pair of legs harvest pollen directly from the stamens. If the pollen ball has grown to a size of about 15 mg a spine located above the tibia detaches it and transports it to the hive (Jean-Prost, 2005).



Figure 6: Pollen Grid Trap Functioning

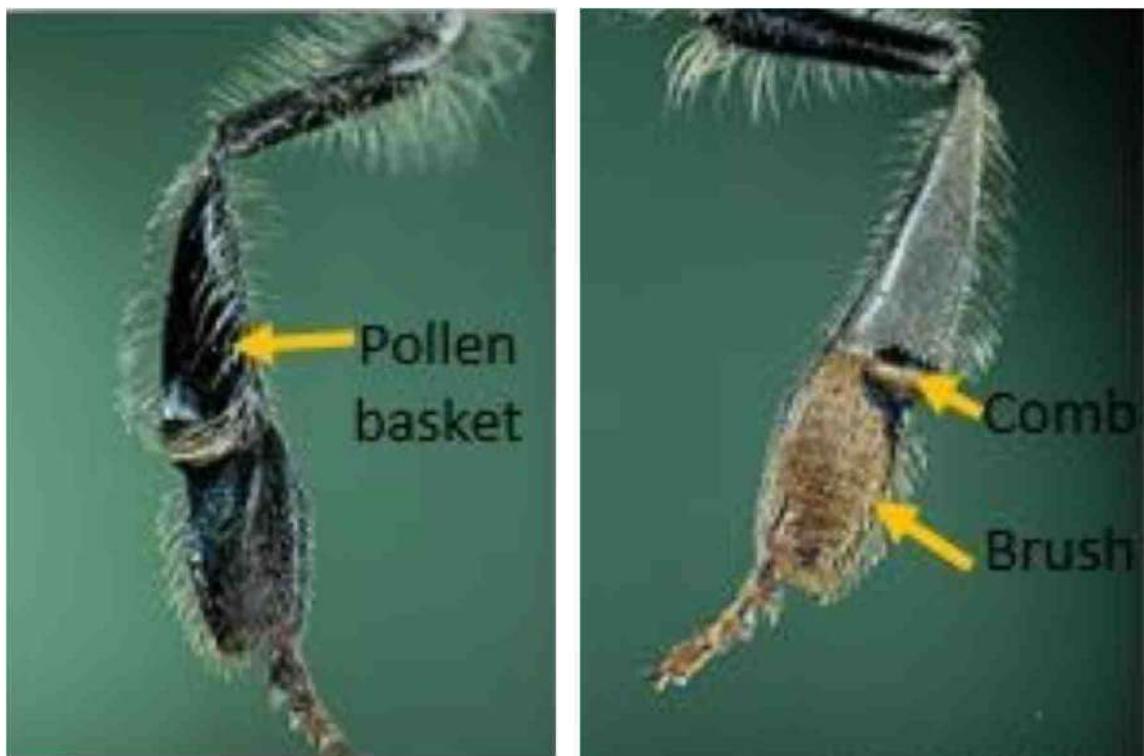


Figure 7: Pollen Basket and Comb Brush

The beekeeper sets up a pollen trap for one day starting at 7-8 A.M. and taking it down around 8-9 P.M. Pollen samples are collected in a plastic bag with the date and weight of the sample written on it. The sample bag is placed in the freezer after it has been collected. In addition, “the sample bag is sent to the lab in a temperature controlled box with ice to preserve the pollen which is extremely sensitive to temperature and humidity changes. The pollen ball composition and percentage of each plant species was examined in the study.



Chapter-7

POLLEN COLLECTION AND BEEHIVE

Pollen Trap:

Pollen traps are used to collect pollen grains in honey bee hives. When worker bees move through the entrance of the nest pollen traps dislodge corbicular pollen loads from the legs of the bees. Pollen traps can efficiently remove large amounts of pollen grains from returning bees with little effort. Pollen traps have been used for decades to remove pollen grains from honey bees in an experimental environment (Al-Tikrity *et al.*, 1972). These pollen traps are very successful at large scale due to the size of honey bee workers. Pollen traps need only slight adjustment after installation and do not necessitate the sacrifice of bees (Goodwin and Perry, 1992). This is accomplished by using screens made of plastic surfaces that dislodge pollen from worker bees hind legs as they return to the hive. These pollen traps remove only a portion of the pollen grains from returning foragers and the various design result in varying pollen collection efficiencies. When the pollen is fully removed from the worker bee legs it falls through a screen and into a storage basin that the bees cannot access where it can then be easily removed by the researcher or scientist with minimal disruption to the beehives (Judd *et al.*, 2020).

Pollen Collection:

The scraping of pollen grains from the legs of worker bees as they enter the beehive is a recent advancement in beekeeping that is based primarily on the core idea of scraping pollen grains from the legs of worker bees as they enter the beehive (Feas *et al.*, 2012). These bees gather pollen grains by mixing sugar derivatives from nectar with their own secretions which attach to pollen grains as well (Cheng *et al.*, 2013). After this step the finished product is packed into the hairs of worker bees hind legs and returned to the colony (LeBlanc *et al.*, 2009). Indoor and outdoor pollen collectors can be used for the commercial pollen grain collection. Depending on the type of hive different variations of these pollen collectors may be used but the principle of pollen extraction remains the same. Pollen carrying honey bees must scrap pollen grains through small openings in pollen collectors whether outdoor or indoor where the pollen grains fall into the prepared drawer. In contrast to indoor pollen collectors the main advantage of outdoor pollen collectors is



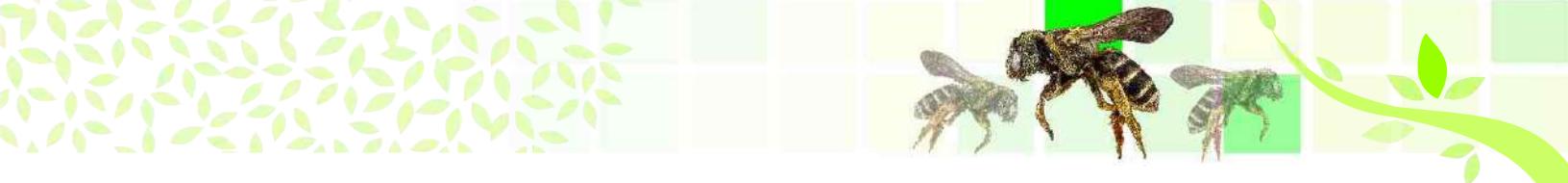
clean pollen but the main disadvantage is small volumes. Since raw pollen grains with a moisture content of 20 per cent are susceptible to microbial spoilage they must be held frozen at -18°C or dried to an moisture content of 7 to 8 per cent and stored in a cold, dark spot. The extract of pollen grains in various solvents and their mixture are prepared for pollen grain analysis. Methanol, ethanol and water are the most widely used solvents in pollen grain analysis (Alicic *et al.*, 2014).

Botanical Origin of Pollen:

Each pollen producing plant species has its own distinct pollen grain based on the botanical origin of the plant species *i.e.* identifying the plants that bees visited for pollen collection. Pollen grains differ from one another in terms of morphological characteristics such as shape, height, openings/apertures and ornamentation as well as colour and appearance. Pollen grains are identified by their colour and other features which are used to determine the genus and species of plants (Bacic, 1995; de Arruda *et al.*, 2013). Analysis of pollen grains allows the identification of the major pollen producing sources mainly used by the bees and the periods of pollen production in the field at a very short time (de Arruda *et al.*, 2013). Microscopic analysis of pollen grains revealed that each pellet of honeybee collected pollen was essentially homogeneous, confirming the findings of Almaraz-Abarca *et al.* (2004) that pollen pellets primarily contain pollen grains from a single species. Research by de Arruda *et al.* (2013) showed that bees use a variety of flora for producing pollen grains and other natural bee products. Bees generally visit the same type of flora to make pollen grains and that pollen is mainly monofloral with a little additions of pollen grains of other species of plants. According to de Arruda *et al.* (2013) pollen grain samples which have amounts exceeding 45 per cent of a botanical taxon in their natural composition can be considered as unifloral pollen. According to Luz *et al.* (2010) the pollen types observed in pollen pellets differ depending on the location where they are provided a factor that is dependent on the surrounding bee pasture in the apiary vegetation as well as the climatic conditions for perfect flowering.

Langstroth Beehive:

Sudarsan *et al.* (2012) found the Langstroth beehive named after its inventor in their observations (U.S. Patent 9300, 1852). Figure 9 depicts a standard beehive which consists of a board on the bottom side to which boxes with the same rectangular cross sections and different heights are stacked. The bottom board has a 2 cm high base three sides on which the boxes are placed and an opening in the front side that serves as an entrance for the honeybees to enter and exit the beehive. It also extends 5 cm in front of the boxes to provide honeybees with a landing platform. Rectangular wooden boxes with no top and bottom surfaces make up the beehive. The lower boxes (brood chambers) are attached first to the top of the bottom board. On the frames of these boxes is the beehive's brood field. Honey supers are the boxes that are mounted on top of the brood chamber and can be deep or shallow in height. The queen excluder a wire mesh that keeps the queen honeybee in the brood chamber and prevents her from laying eggs in the honey supers separates these chambers. In summer a typical beehive would have one or two brood chambers and one to ten honey supers. Honey supers and brood chambers of beehives have removable frames Figure 10(a) which are supported on notches running in the front side and back sides of the hive body near the top. The Langstroth beehive design also allows beekeepers to extract honey from super frames and remove damaged frames without disrupting the rest of the hives activities. It is possible to remove the frames individually depending on the health of the brood using this design. The Langstroth beehive's universal appeal, durability (150 years) and success are due to these characteristics. Each frame has a wax compact base with hexagonal ridges known as comb surfaces. Honey bees use beeswax on the comb



surface to build hexagonal shaped cells. The primary role of queen bees is to stay in the brood chamber and complete the egg laying process in the comb cells. Worker bees cap these comb cells with formed pupae or cured honey using beeswax at the same time. The honey supers combs serve as a storage area for the nectar/honey gathered by the bees. Over the summer the honey supers combs will easily fill up with honey so beekeepers sometimes replace the honey super with an empty one. The comb surfaces are perpendicular to the inlet at the bottom side of the board in commonly used Langstroth hives which have 8-12 frames. The frames are separated by an equivalent distance known as "bee room" which is between 4.5 mm and 8 mm. Honey bees glue the two opposite combs together if the spacing is less than 4.5 mm whereas wider gaps of more than 8 mm are filled with wax comb by the bee. The honey bees will access the intermediate spacing between the combs and assume in our model a bee space value of 8 mm. Just one inlet was present in the original Langstroth beehive which was situated at the bottom of the brood chamber. Many of the beekeepers have changed the design over the years to include additional openings. The Langstroth hive is known for "bee space" where the spacing between frames is large enough that *Apis mellifera* bees tend to avoid adding propolis to fill gaps and small enough to avoid wax comb in between the frames. For these reasons the Langstroth hive is the default standard for much of the world. It is the most common hive in the US and for migratory beekeepers in India (Jonathan, 2016).

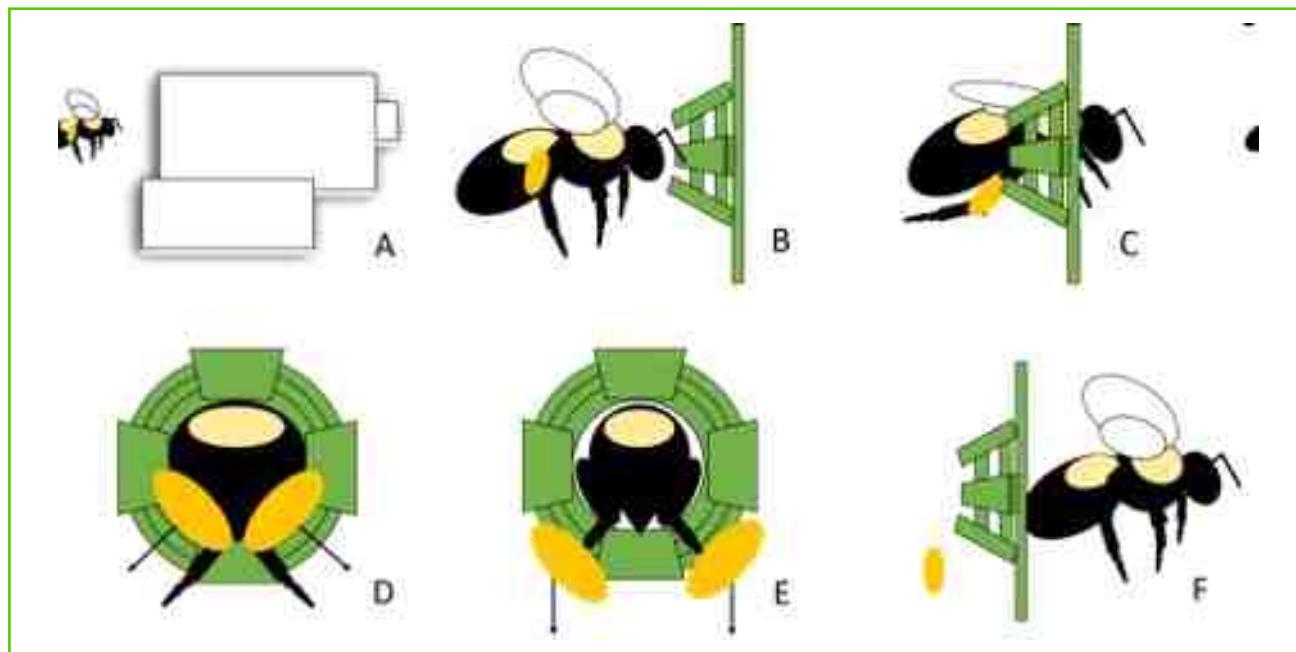


Figure 8: Removal of Corbicula Pollen Loads from Legs of Workers (Bumble Bees, Judd et al., 2020)

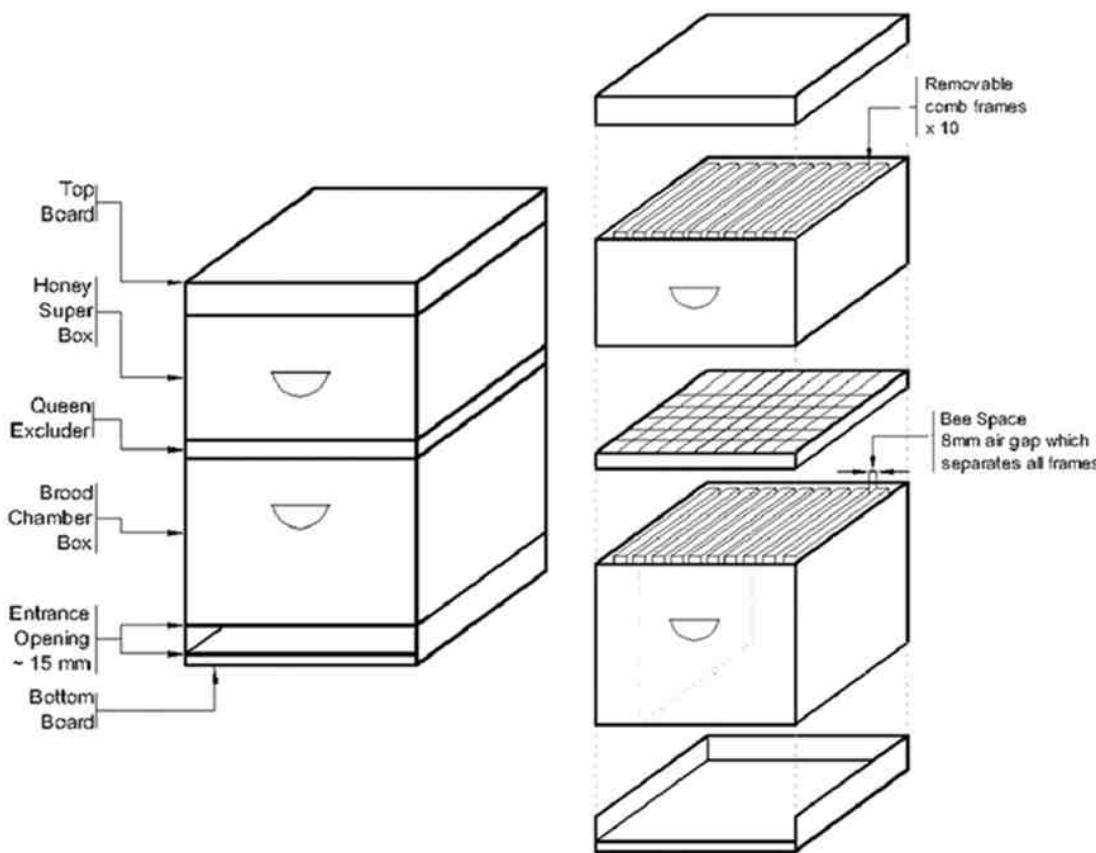


Figure 9: Langstroth Beehive with One Brood Chamber and One Honey Super (Sudarsan *et al.*, 2012)

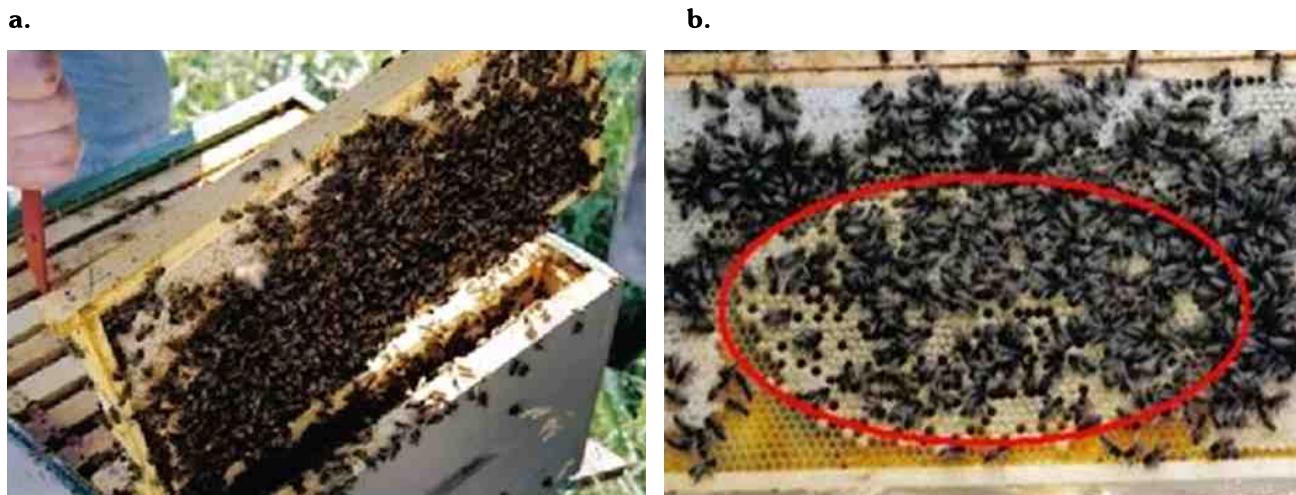
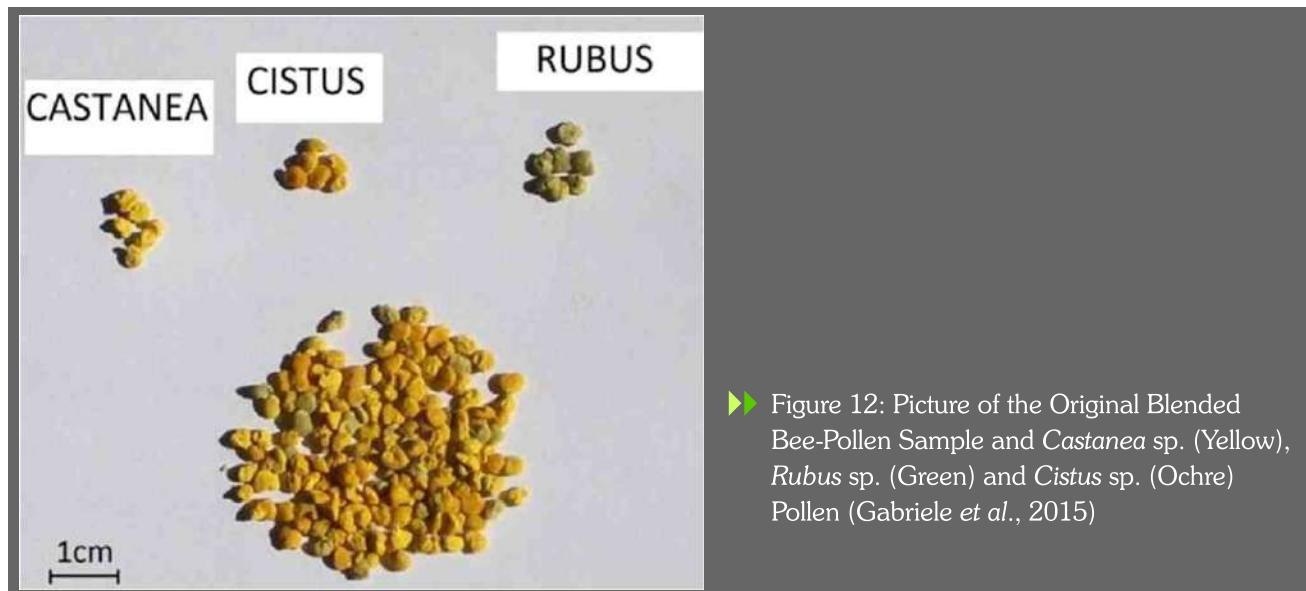


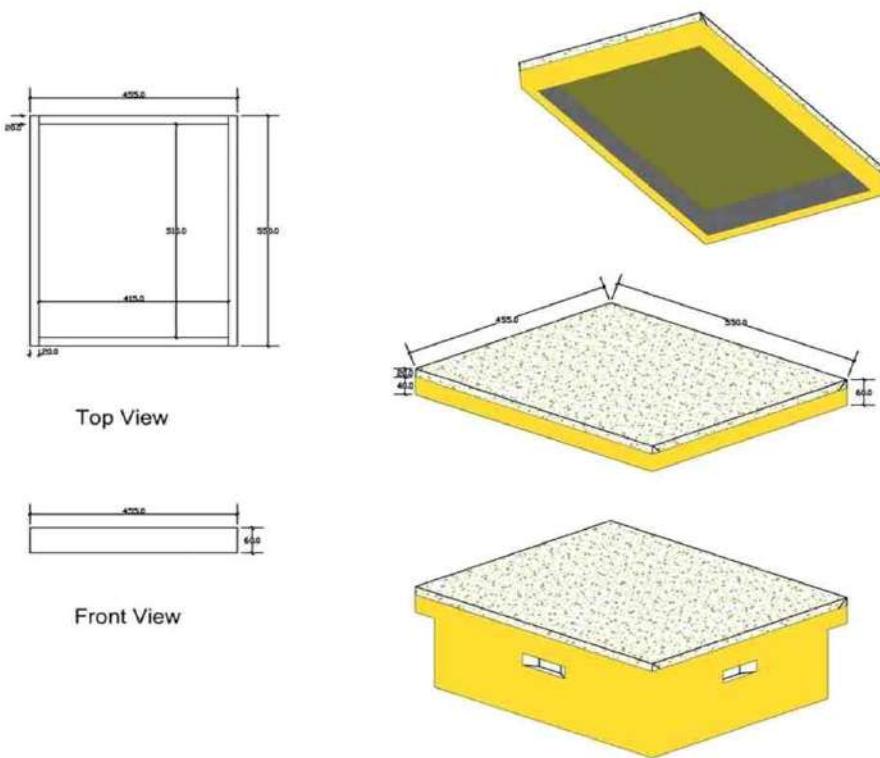
Figure 10: (a) Removable Frame of a Beehive Brood Chamber Showing the Cluster of Bees Sitting on the Comb (b) Location and Shape of Brood (Sudarsan *et al.*, 2012)



Figure 11: Bee Pollen Collection in the Presence of Honourable Governor of Uttarakhand Smt. Baby Rani Maurya and other Dignitaries at Rajbhawan, Dehradun during Spring Festival-2021 Organised by the Department of Horticulture and Food Processing, Government of Uttarakhand



► Figure 12: Picture of the Original Blended Bee-Pollen Sample and *Castanea* sp. (Yellow), *Rubus* sp. (Green) and *Cistus* sp. (Ochre) Pollen (Gabriele et al., 2015)



► Figure 13: Different Views of Top Hive Cover for Standard Langstroth Hive (Wakjira and Zur, 2018)

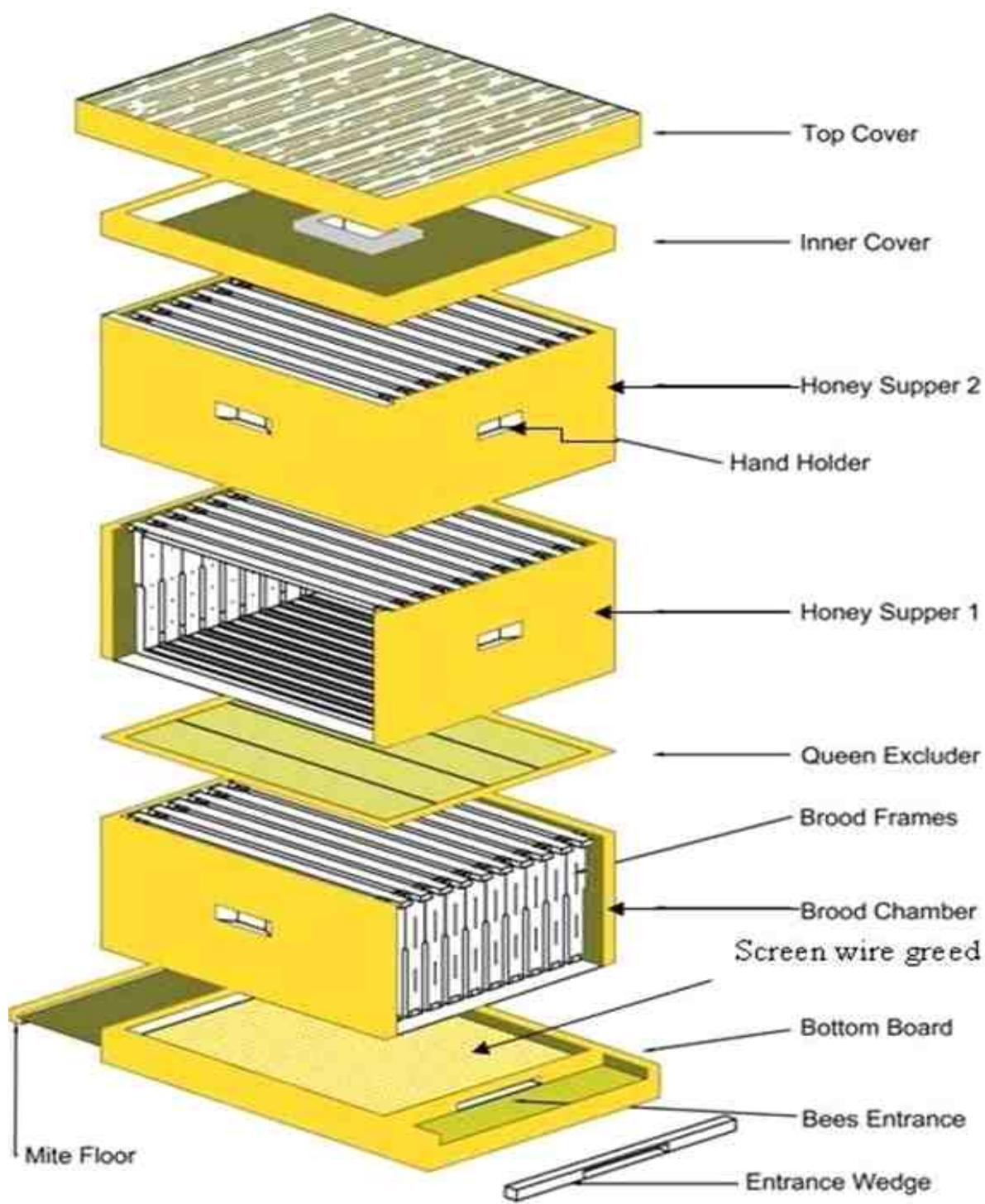
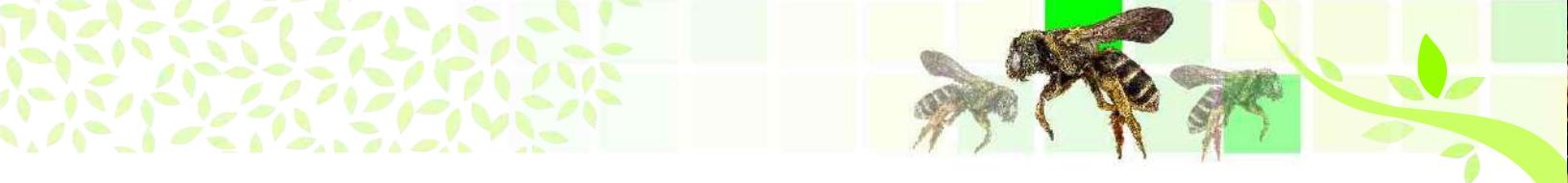


Figure 14: A Complete Assembled Beehive Sketch (Wakjira and Zur, 2018)



Chapter-8

PROCESSING

Bee Pollen Processing:

The word pollen which comes from a Latin word that means fine powder (flour) was introduced in the 17th century and has often been referred to as "meal" for centuries (Bogdanov, 2017). Hence, it excessively benefits the insect's diet so bee pollen has become key component of animal feed in terms of protein source and also a quality natural substitute for antibiotics (Abdelnour *et al.*, 2019). "The first review on regarding pollen as a food for humans was probably presented in the 70s analysing the currently available data concluded that the pollen is taken as a dietary supplement for weight improvement and for the prevention of certain respiratory infections by some athletes from different countries (Bogdanov, 2017). "In addition to it they also emphasized on the fact that regular consumption of pollen enriched honey benefits extends the lives of some residents of Ukraine and Russia linked to it" (Stanley and Linskens, 1974). Following this an advance progress has been made about the pollen application as a dietary supplement has been made due to which many research articles have been published in an increased number.

In today's time pollen is commonly considered as the "only perfectly complete food" (Ares *et al.*, 2018) and "the world's best food product" (Kieliszek *et al.*, 2018). "Because of its high concentration of proteins, carbohydrates and lipids pollen is an excellent source of natural energy substitute. It is also high in nutritional content, controls various biochemical processes and enhances the immune and physiological systems of the body" (Ares *et al.*, 2018 and Li *et al.*, 2018). Pollen is an ample source of numerous essential compounds such as vitamins, carotenoids, minerals and polyphenols (Antonelli *et al.*, 2019; De Arruda *et al.*, 2013; Margaoan *et al.*, 2014). This makes it valuable source for use in the diets of children and adults suffering from certain avitaminoses and loss of appetite. Pollen possesses antioxidant, antibacterial, anti carcinogenic, hepatoprotective and cardioprotective properties due to the existence of various active natural metabolites, primarily vitamins, carotenoids and polyphenols. Antonelli *et al.*(2019) stated that pollen is considered as an important tool for the therapeutic treatment of various non allergic diseases. "It was also discovered that flower pollen extracts can be used as a complementary medicine for the treatment of benign prostatic hyperplasia, chronic prostatitis and vasomotor symptoms in women but clinical effectiveness needs to be further investigated first" (Antonelli *et al.*, 2019). "Hence, various pollen products are available in the form of granules, capsules, tablets, pellets and powders in the market" (Li *et al.*, 2018).



Pollen intake should be between 20 and 40 g a day for an adult on a regular basis. According to Kieliszek *et al.* (2018) fresh or dried pollen grains have a hard shell (intine and exine) that affects the “penetration of digestive enzymes into pollen pellets as well as the absorption of essential nutrients by the human digestive tract”. The average degree of digestibility of carbohydrates was 4 per cent for carbohydrates and 53 per cent for proteins, based on the pollen origin (Komosinska *et al.*, 2015). “When pollen grains are ground and dissolved in warm water the accessibility of nutrients as well as the digestibility and functionality of the pollen grains increases to 60-80 per cent” (Kieliszek *et al.*, 2018). Recently, pollen nowadays considered as a “feed ingredient and functional food” due to the rise in awareness among the consumers that the consumption of functional foods can boost up their health. A large number of fermented pollen based food products have been developed (Utoiu *et al.*, 2018; Couto *et al.*, 2006) including bee-collected pollen-based baking products (Conte *et al.*, 2020), confectionery items (Krystyan *et al.*, 2015), juice (Zuluaga *et al.*, 2016) and meat (Almeida *et al.*, 2017) food products. “The use of bee pollen as a feed additive for livestock and poultry reveals that bee pollen boosts animal development, reproduction and immunity” (Abdelnour *et al.*, 2019). Pollen can contain anti-nutritional substances such as allergens, pyrrolizidine alkaloids, poisonous and potentially toxic components and mycotoxins despite all of the advantages that can be derived from pollen as a practical food product (Krocko *et al.*, 2012). “For a specific group of consumers some allergic reactions will be expressed if they are sensitive to the presence of pollen”. Pollen related food allergy (PRFA) is typically obtained through the sudden production of oral allergy symptoms following food intake with random resolution within 10-30 minutes and heating, baking or frying will render certain foods tolerable to the majority of possibly allergic individuals (Worm *et al.*, 2014) but PRFA should not be ignored.

Routes of Administration and Dosing:

In adults, 20-40 g dose is applied every day for curative purposes. Since there are 5-7 g of pollen in a teaspoon, one dosage for an adult is 3-5 teaspoons and 1-2 teaspoons for children, pollen should be taken three times per day before feeding. Treatment lasts 1-3 months and can be replicated 2-4 times a year. Winter and spring as well as summer are the most convenient times for recovery. Besides other medications a smaller dose of pollen is used in the combination therapy and in various chronic diseases (Bogdanov, 2014).

Bee Bread:

Bee bread is formed by adding honey and digestive enzymes to bee pollen during its storage in the honey comb and by fermentation of lactic acid. The titration acidity increases during the conversion of bee pollen into bee bread while the content in sitosterol and vitamins decreases. The composition of bee bread has a major impact when it comes to the flora in the colonies region; it is similar to bee pollen and varies by botanical origin (Margaoan *et al.*, 2019). Bee bread is normally offered in lower amounts or for a shorter period of time than pollen because of its stronger action. Romanian researchers used 30 g of bee pollen for three months and 30 g of bee bread for one month to achieve the same outcomes in the treatment of chronic hepatitis. Pollen grains are shredded by grinding or soaking in warm water to improve the organism digestibility. As a result, pollen grains swell and break after 2-3 hours releasing their values. For this milk, meat and vegetable juices were also used. Honey, butter, cottage cheese, yoghurt, preserves, glucose and other items can be combined with ground pollen powder in a ratio of 1:1 to 1:4 with honey, butter, yoghurt, jams, cottage cheese and other products. Then one teaspoon of mixed pollen should be taken three times a day. Enzymatic pollen on the other hand is prescribed for the treatment of a variety of diseases. “But after



mechanical shredding in the powder form or natural release, the availability of biological pollen increases upto 60-80 per cent" (Bogdanov, 2014; Rimpler, 2003).

The Application of Pollen as a Functional Food Ingredient:

Before considering the pollen as a functional food ingredient it is very necessary to study and understand the appropriate ways for storing and preservation of pollen in order to preserve all its nutrients. "As it is very well known that inadequate storage leads to decrease in pollen's viability and germination ability and also makes it affected by several factors such as humidity, temperature, gas atmosphere and oxygen pressure" (Stanley and Linskens, 1974). Due to all these reasons, it is very important to make a suitable preservation process with regular quality control that must be conducted throughout the whole time.

The Effect of Drying Techniques and Storage Conditions on the Quality of Bee Collected Pollen:

It is well known that freshly harvested pollen produces high levels of moisture, ranging from 20 per cent to 30 per cent which promotes the rapid growth of microorganisms, chemical and enzymatic reactions decreasing the shelf life and pollen utilisation capacity. The water activity of pollen required for consumption according to Gonzalez *et al.* (2005) should be in the range of 0.261-0.280 but it is also observed that the drying phase and storage conditions affect the consistency of bee-collected pollen.

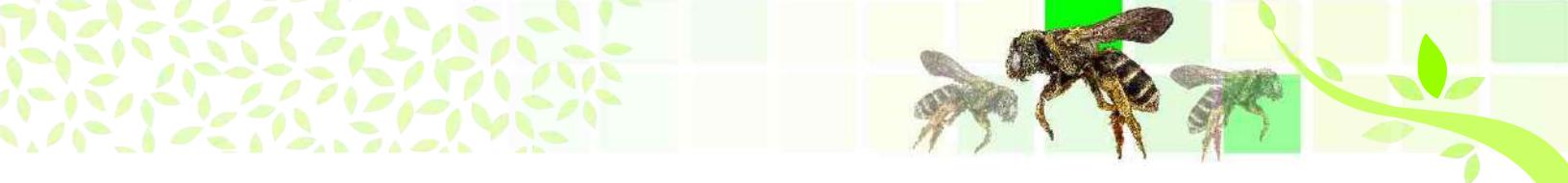
The Influence of Drying Techniques on the Quality of Bee Collected Pollen:

For proper storage of freshly collected pollen different drying techniques like hot-air drying, freeze-drying, microwave drying, vacuum drying and microwave assisted vacuum drying are used out of which freeze-drying and hot-air drying are most commonly applied. Pollen drying with hot air is usually recommended at temperatures between 40°C and 60°C though higher temperatures disrupt its physicochemical properties, morphological structures and organoleptic characteristics (Isik *et al.*, 2019). Collin *et al.* (1995) found that drying pollen at 30°C or for a brief period at 40°C can be efficient in preventing the formation of dimethyl sulphide (pollen is high in 5J-methylmethionine), undesirable aldehydes and furan compounds, as well as the loss of desirable monoterpenic compounds.

"Freeze drying on the other hand is considered an effective process for handling commercially collected pollen since the individual compounds contained in the pollen are often held within the range needed to standardise commercial pollen as a marketable commodity" (Dominguez-Valhondo *et al.*, 2011). It was seen that the freeze dried pollen had same characteristics to the fresh one as the amount of fructose, glucose, sucrose, dietary fibre and protein were unchanged, whereas the lipid content was increased but on the other hand the amount of free amino acids and bioactive compounds was decrease during the drying process especially in the hot-air-dried samples than in the lyophilized ones.

Fermented Bee Collected and Floral Pollen Based Products:

Fermentation use in food production has been recorded for the entirety of human history. Many items that depend on fermentation such as bread, cheese, yoghurt, wine, soy sauce and fermented sausages are important for human nutrition. Fermentation increases the shelf life of food while also increasing its microbiological stability. It also



increases the digestibility and nutritional properties of certain foods contributes to the development of textures and distinct flavours and in some cases fermented foods can be claimed to have probiotic properties (Yans *et al.*, 2019). Pollen has always been a priority for food science but lately there has been a lot more focus on providing improved final goods and one of the key areas of study is fermentation as an effective method for producing bee collected and floral pollen based products. More and more products are being procured by pollen fermentation or other raw materials to which pollen has been added. The following are some main conclusions about the impact of bee collected/floral pollen on final product quality:

1. Pollen fermentation with the kombucha/SCOBY consortium was found to significantly increase the bioavailability of the bioactive compounds found in pollen resulting in the development of kombucha health-related components and a product with a mild antitumor impact on Caco-2 cells. According to Utoiu *et al.* (2018) pollen fermentation by the kombucha/SCOBY consortium increases the release of polyphenols and flavonoids from pollen into the liquid process of the fermented substance as well as the additional deposition of organic acids especially hydroxy acids such as citric, gluconic, lactic acid and short-chain fatty acids like acetic, propionic and butyric acids.
2. Pollen addition (ranging from 10 to 50 mg/L) during the production of honey wine increased the ethanol content (from $11.74\% \pm 0.06\%$ to $12.39\% \pm 0.12\% v/v$), which may be directly related to the application of pollen as a fermentation activator (Roldan *et al.*, 2011).
3. Karabagias *et al.* (2018) studied that in comparison to the conventional yoghurt, bee collected pollen enriched with yoghurt enhance better antioxidant capacity an increase in polyphenolic content and significantly better sensory properties. Hence, the addition of pollen gives out positive effects on the textural and techno functional properties of yogurt such as increased gel strength and decreased syneresis (Atallah and Morsy, 2017).
4. Krystyjan *et al.* (2015) published a study to find the best recipe for making biscuits with added bee collected pollen as well as physical, chemical and sensory characterization. The results showed that the addition of bee-collected pollen significantly increased the content of sugars, proteins, ash, fibers, polyphenols and the antioxidant potential of the final products while it had no effect on their lipid content.
5. Solgajova *et al.* (2014) reviewed that the addition of pollen subjected to its concentration and origin increased the content of the reducing sugars, protein, ash content and the antioxidant activity of the final product. Technological parameters such as the diameter and the weight of the cookies were increased while the thickness of the product decreased with the gradual addition of pollen. The cookies were characterized by pleasant and easy chewiness with a delicate taste.
6. In order to improve the extractability and abundance of pollen bioactive compounds, Zuluaga *et al.* (2016) established high pressure processing (HPP) treatments as a method for inactivating microorganisms. However, a particular area of interest was the impact of the treatment on the pineapple juice-based beverage matrix to which pollen was added. According to the results, HPP treatment improved the extractability of some bioactive compounds present in the bee collected pollen grains. Therefore, an increase in the total carotenoid content (TCC), the total phenolic content (TPC) and the antioxidant capacity (FRAP) in the bee-collected pollen-based beverage was confirmed as a consequence of the HPP treatments. Also, the treatments showed the effectiveness of the inactivation of microorganisms.
7. Thakur and Nanda (2019) made a pollen rich milk powder from vacuum dried bee pollen. For the purposes of the process, optimization and the definition of optimal parameters, a response surface methodology based on the results determined for physicochemical and functional properties was applied. The resulting powder



proved to be a significant source of polyphenols giving it the ability to be used in various industries to make healthier food products.

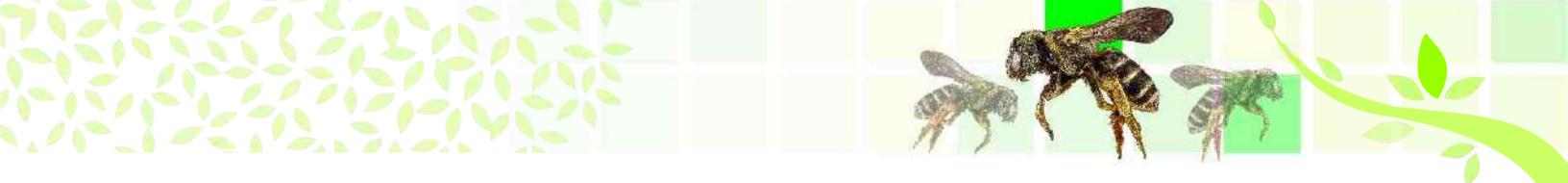
8. Vamanu *et al.* (2006) produced a pollen-based probiotic product by inoculating the grounded or ungrounded pollen and honey *Lactobacillus acidophilus* strains as a medium.
9. Abdelhamid and Elbayoumi (2017) investigated the effect of bee-collected pollen supplementation (@ 0.5; 1.0; 1.5 and 2.0 per cent) on the antibacterial, sensory and physicochemical properties of white cheese made from a mixture of cow and camel milk as well as the antioxidant function. According to the findings, adding bee collected pollen to cheese increased polyphenol content, fat and protein content and antioxidant activity and reducing power assay from 30.83 to 98.37 per cent inhibition). When compared to a control sample with up to 1 per cent bee collected pollen added no differences in sensory properties were found.

Spring Festival 2021 (Uttarakhand):

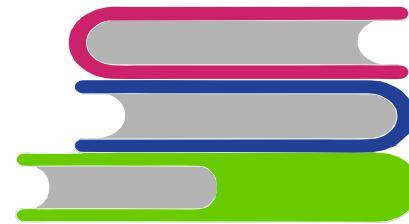
Bee pollen collection and extraction of honey was also done in the presence of the Honourable Governor of Uttarakhand Smt. Baby Rani Maurya at Raj Bhavan, Dehradun during Spring Festival-2021 (Uttarakhand) that was organised by the Department of Horticulture and Food Processing, Government of Uttarakhand on 13th of March, 2021 and 14th of March, 2021 (Figure 11).

Source of Employment:

Honey bee Pollen and Propolis collection has a tremendous scope in Uttarakhand as it will boost the income of farmers. Uttarakhand has places like valley of flowers and has a huge floral wealth these flowers can serve as a source of organic bee pollen. Farmers of Uttarakhand will be trained for bee pollen and its collection. Bee pollen collection will also serve as a source of employment to young entrepreneurs and will double the income of farmers. Pollen and Propolis is effective against COVID-19 so it should be promoted for its health benefits. Moreover, pollen collection techniques and its detailed information to the farmers will be provided by the Department of Horticulture and Food Processing, Government of Uttarakhand.



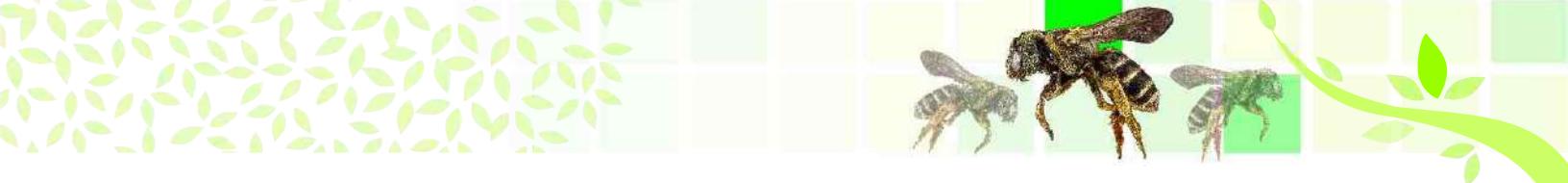
GLOSSARY



Abdomen	: the posterior or third region of the body of a bee enclosing the honey stomach, true stomach, intestine, sting and reproductive organs.
Absconding swarm	: an entire colony of bees that abandons the hive because of disease, wax moth or other maladies.
Adulterated honey	: any product labeled "Honey" or "Pure Honey" that contains ingredients other than honey but does not show them on the label. (Suspected mislabeling should be reported to the Food and Drug Administration.)
After swarm	: a small swarm, usually headed by a virgin queen, which may leave the hive after the first or prime swarm has departed.
Alighting board	: a small projection or platform at the entrance of the hive.
American foulbrood	: a brood disease of honey bees caused by the spore-forming bacterium, <i>Bacillus larvae</i> .
Anaphylactic shock	: constriction of the muscles surrounding the bronchial tubes of a human, caused by hypersensitivity to venom and resulting in sudden death unless immediate medical attention is received.
Anaerobic	: living, active, occurring or existing in the absence of free oxygen.
Anemophilous	: pollinated by the wind.
Angiogenesis	: physiological process through which new blood vessels form from pre-existing vessels, formed in the earlier stage of vasculogenesis.
Anther	: the part of a flower at the top of a stamen that produces pollen.
Antiatherosclerotic	: an agent that prevents or counteracts atherosclerosis.
Antioxidants	: substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures.



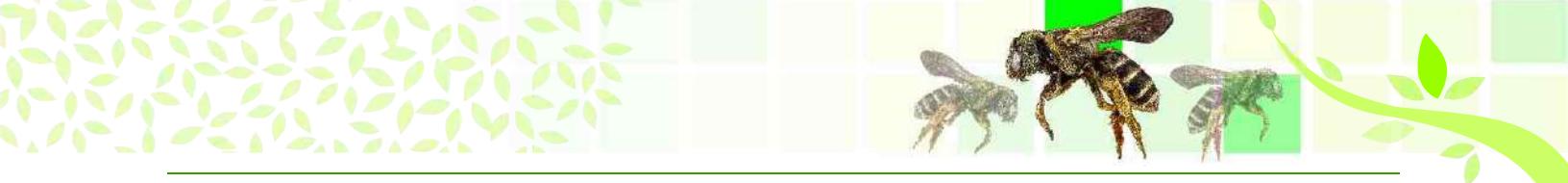
Annual	: a plant that lives only one growing season.
Apiary	: colonies, hives and other equipment assembled in one location for beekeeping operations; bee yard.
Apiculture	: the science and art of raising honey bees.
Apitherapy	: a type of alternative therapy that uses products that come directly from honeybees.
<i>Apis mellifera</i>	: scientific name of the honey bee found in the United States.
Attenuation	: a gradual loss of flux intensity through a medium.
Bacillus larvae	: the bacterium that causes American foulbrood.
Bee blower	: an engine with attached blower used to dislodge bees from combs in a honey super by creating a high-velocity, high-volume wind.
Bee bread	: a mixture of collected pollen and nectar or honey, deposited in the cells of a comb to be used as food by the bees.
Bee brush	: a brush or whisk broom used to remove bees from combs.
Bee escape	: a device used to remove bees from honey supers and buildings by permitting bees to pass one way but preventing their return.
Beehive	: a box or receptacle with movable frames, used for housing a colony of bees.
Bee metamorphosis	: the three stages through which a bee passes before reaching maturity: egg, larva and pupa.
Bee space	: 1/4 to 3/8 inch space between combs and hive parts in which bees build no comb or deposit only a small amount of propolis.
Bees wax	: a complex mixture of organic compounds secreted by special glands on the last four visible segments on the ventral side of the worker bee's abdomen and used for building comb. Its melting point is from 143.6 to 147.2 degrees F.
Bee tree	: a tree with one or more hollows occupied by a colony of bees.
Bee veil	: a cloth or wire netting for protecting the beekeeper's head and neck from stings.
Bee venom	: the poison secreted by special glands attached to the stinger of the bee.
Benzaldehyde	: a volatile, almond smelling chemical used to drive bees out of honey supers.
Biennial	: a plant that lives through two growing seasons before it sets seed and dies.



Boardman feeder	: a device for feeding bees in warm weather, consisting of an inverted jar with an attachment allowing access to the hive entrance.
Bottom board	: the floor of a beehive.
Brace comb	: a bit of comb built between two combs to fasten them together, between a comb and adjacent wood, or between two wooden parts such as top bars.
Brassinosteroids	: a class of polyhydroxysteroids that have been recognized as a sixth class of plant hormones.
Brood	: bees not yet emerged from their cells: eggs, larvae and pupae.
Brood chamber	: the part of the hive in which the brood is reared; may include one or more hive bodies and the combs within.
Capped brood	: pupae whose cells have been sealed with a porous cover by mature bees to isolate them during their non feeding pupal period; also called sealed brood.
Capping melter	: melter used to liquefy the wax from cappings as they are removed from honey combs.
Cappings	: the thin wax covering of cells full of honey; the cell coverings after they are sliced from the surface of a honey filled comb.
Cardiovascular	: related to the circulatory system, which comprises the heart and blood vessels and carries nutrients and oxygen to the tissues of the body and removes carbon dioxide and other wastes from them.
Castes	: the three types of bees that comprise the adult population of a honey bee colony: workers, drones and queen.
Cell	: the hexagonal compartment of a honey comb.
Cell cup	: base of an artificial queen cell, made of beeswax or plastic and used for rearing queen bees.
Chilled brood	: immature bees that have died from exposure to cold; commonly caused by mismanagement.
Chromatography	: a laboratory technique for the separation of a mixture.
Chunk honey	: honey cut from frames and placed in jars along with liquid honey.
Clarifying	: removing visible foreign material from honey or wax to increase its purity.
Cluster	: a large group of bees hanging together, one upon another.
Colony	: the aggregate of worker bees, drones, queen and developing brood living together as a family unit in a hive or other dwelling.



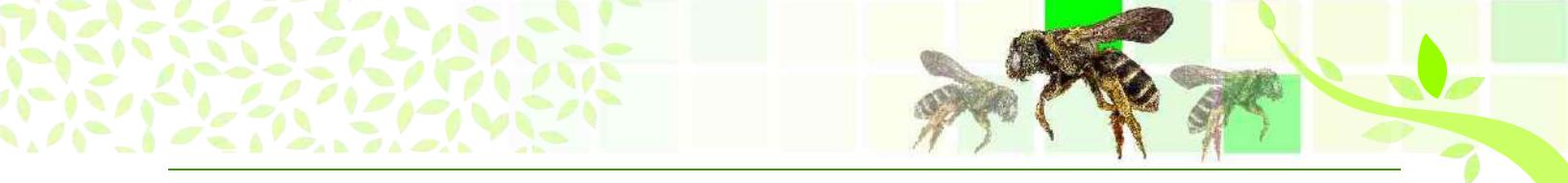
Comb	: a mass of six-sided cells made by honey bees in which brood is reared and honey and pollen are stored; composed of two layers united at their bases.
Comb foundation	: a commercially made structure consisting of thin sheets of bees wax with the cell bases of worker cells embossed on both sides in the same manner as they are produced naturally by honey bees.
Comb honey	: honey produced and sold in the comb, in either thin wooden sections (4 x 4 inches or 4 x 5 inches) or circular plastic frames.
Creamed honey	: honey which has been allowed to crystallize, usually under controlled conditions, to produce a tiny crystal.
Crimp-wired	: comb foundation into which crimp wire is embedded vertically during foundation manufacture.
Cut-comb honey	: comb honey cut into various sizes. The edges drained and the pieces wrapped or packed individually.
Cytotoxicity	: quality of being toxic to cells.
Swarm catcher	: a hive placed to attract stray swarms.
Dividing	: separating a colony to form two or more units.
Division board feeder	: a wooden or plastic compartment which is hung in a hive like a frame and contains sugar syrup to feed bees.
Docking	: a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.
Double Screen	: a wooden frame with two layers of wire screen to separate two colonies within the same hive or above the other. An entrance is cut on the upper side and placed to the rear of the hive for the upper colony.
Drawn combs	: combs with cells built out by honey bees from a sheet of foundation.
Drifting of bees	: the failure of bees to return to their own hive in an apiary containing many colonies. Young bees tend to drift more than older bees and bees from small colonies tend to drift into larger colonies.
Drone	: the male honey bee.
Drone comb	: comb measuring about four cells per linear inch that is used for drone rearing and honey storage.
Drone layer	: an infertile or unmated laying queen.
Dwindling	: the rapid dying off of old bees in the spring; sometimes called spring dwindling or disappearing disease.



Dysentery	: an abnormal condition of adult bees characterized by severe diarrhea and usually caused by starvation, low-quality food, moist surroundings or nosema infection.
Endogenous	: A substances and processes are those that originate from within a system such as an organism, tissue, or cell.
European foulbrood	: an infectious brood disease of honey bees caused by <i>Streptococcus pluto</i> .
Extracted honey	: honey removed from the comb by centrifugal force.
Fermentation	: a chemical breakdown of honey, caused by sugar-tolerant yeast and associated with honey having a high moisture content.
Fertile queen	: a queen, which has been inseminated, naturally or artificially and can lay fertilized eggs.
Fertilization	: the fusion of haploid gametes, egg and sperm to form the diploid zygote.
Field bees	: worker bees at least three weeks old that work in the field to collect nectar, pollen, water and propolis.
Frame	: four pieces of wood designed to hold honey comb, consisting of a top bar, a bottom bar and two end bars.
Fructose	: the predominant simple sugar found in honey; also known as levulose.
Foraging	: the act of gathering wild food for free.
Fumidil-B	: the trade name for Fumagillin, an antibiotic used in the prevention and suppression of nosema disease.
Fume board	: a rectangular frame, the size of a super, covered with an absorbent material such as burlap, on which is placed a chemical repellent to drive the bees out of supers for honey removal.
Glycoproteins	: proteins which contain oligosaccharide chains (glycans) covalently attached to amino acid side-chains.
Glucids	: a polyhydroxylated aldehydes or ketones, their derivatives and polymers.
Grafting	: removing a worker larva from its cell and placing it in an artificial queen cup in order to have it reared into a queen.
Grafting tool	: a needle or probe used for transferring larvae in grafting of queen cells.
Granulation	: the formation of sugar (dextrose) crystals in honey.
Hemophagocytic lymphohistiocytosis	: a severe systemic inflammatory syndrome that can be fatal. This syndrome can sometimes occur in normal people with medical problems that can cause a strong activation of the immune system, such as infection or cancer.



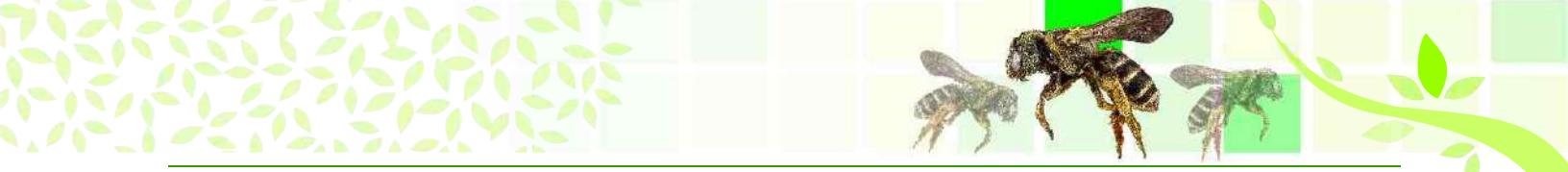
Hepatoprotective	: the ability of a chemical substance to prevent damage to the liver.
Hive	: a man-made home for bees.
Hive body	: a wooden box which encloses the frames.
Hive stand	: a structure that supports the hive.
Hive tool	: a metal device used to open hives, pry frames apart and scrape wax and propolis from the hive parts.
Honey	: a sweet viscous material produced by bees from the nectar of flowers, composed largely of a mixture of dextrose and levulose dissolved in about 17 per cent water; contains small amounts of sucrose, mineral matter, vitamins, proteins and enzymes.
Honeydew	: a sweet liquid excreted by aphids, leaf hoppers, and some scale insects that is collected by bees, especially in the absence of a good source of nectar.
Honey extractor	: a machine which removes honey from the cells of comb by centrifugal force.
Honey flow	: a time when nectar is plentiful and bees produce and store surplus honey.
Honey gate	: a faucet used for drawing honey from drums, cans or extractors.
Honey house	: building used for extracting honey and storing equipment.
Honey pump	: a pump used to transfer honey from a sump or extractor to a holding tank or strainer.
Honey stomach	: an organ in the abdomen of the honey bee used for carrying nectar, honey or water.
Honey sump	: a clarifying tank between the extractor and honey pump for removing the coarser particles of comb introduced during extraction.
Hypopharyngeal gland	: a pair of long glands coiled in the sides of the head of worker honey bee.
Immunomodulatory	: modulation of the immune system.
Increase	: to add to the number of colonies, usually by dividing those on hand.
Inhibitory	: hindering or preventing an action
Inner cover	: a lightweight cover used under a standard telescoping cover on a beehive.
Instrumental insemination	: the introduction of drone spermatozoa into the genital organs of a virgin queen by means of special instruments.
Invertase	: an enzyme produced by the honey bee which helps to transform sucrose to dextrose and levulose.
Larva (plural, larvae)	: the second stage of bee metamorphosis; a white, legless, grub like insect.



Laying worker	: a worker which lays infertile eggs, producing only drones, usually in colonies that are hopelessly queen less.
Macrophage	: a type of phagocyte, which is a cell responsible for detecting, engulfing and destroying pathogens and apoptotic cells.
Mating flight	: the flight taken by a virgin queen while she mates in the air with several drones.
Mead	: honey wine.
Migratory beekeeping	: the moving of colonies of bees from one locality to another during a single season to take advantage of two or more honey flows.
Modulation	: process of converting data into radio waves by adding information to an electronic or optical carrier signal
Mutation	: a change that occurs in our DNA sequence, either due to mistakes when the DNA is copied or as the result of environmental factors.
Nectar	: a sweet liquid secreted by the nectaries of plants; the raw product of honey.
Nectar Flow	: a time when nectar is plentiful and bees produce and store surplus honey.
Nectar Guide	: color marks on flowers believed to direct insects to nectar sources.
Neuroprotective	: relative preservation of neuronal structure and/or function.
Nosema	: a disease of the adult honey bee caused by the protozoan <i>Nosema apis</i> .
Nucleus (plural, nuclei)	: a small hive of bees, usually covering from two to five frames of comb and used primarily for starting new colonies, rearing or storing queens also called 'nue.'
Nurse bees	: young bees, three to ten days old, which feed and take care of developing brood.
Observation hive	: a hive made largely of glass or clear plastic to permit observation of bees at work.
Oligoelements	: a mineral present in the body in a very small quantity yet indispensable to its functioning.
Out-apriary	: an apiary situated away from the home of the beekeeper.
Oxidative Stress	: a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.
Package bees	: a quantity of adult bees (2 to 5 pounds), with or without a queen, contained in a screened shipping cage.



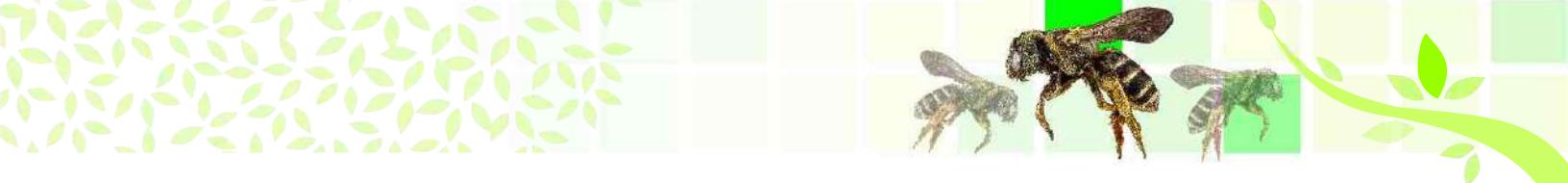
Paralysis	: a virus disease of adult bees which affects their ability to use legs or wings normally.
Parthenogenesis	: the development of young one from unfertilized eggs. In honey bees, the unfertilized eggs produce drones.
PDB (Paradichlorobenzene)	: crystals used to fumigate combs against wax moth.
Piping	: a series of sounds made by a queen, frequently before she emerges from her cell.
Phytochemicals	: compounds that are produced by plants ("phyto" means "plant"). They are found in fruits, vegetables, grains, beans and other plants.
Phytophagous	: insects that feed on green plants.
Play flight	: short flight taken in front of or near the hive to acquaint young bees with their immediate surroundings; sometimes mistaken for robbing or preparation for swarming.
Pleiotropic effects	: a single gene affecting multiple systems or determining more than one phenotype, lately statin pleiotropy is referred as statins exerting multiple pharmacological activities.
Pollen	: the male reproductive cell bodies produced by anthers of flowers, collected and used by honey bees as their source of protein.
Pollen basket	: a flattened depression surrounded by curved spines or hairs, located on the outer surface of the bee's hind legs and adapted for carrying pollen gathered from flowers or propolis to the hive.
Pollen cakes	: moist mixtures of either pollen supplements or substitutes fed to the bees in early spring to stimulate brood rearing.
Pollen substitute	: any material such as soybean flour, powdered skim milk, brewer's yeast, or a mixture of these used in place of pollen to stimulate brood rearing.
Pollen supplement	: a mixture of pollen and pollen substitutes used to stimulate brood rearing in periods of pollen shortage.
Pollen trap	: a device for removing pollen loads from the pollen baskets of incoming bees.
Pollination	: the transfer of pollen from the anthers to the stigma of flowers.
Pollinator	: the agent that transfers pollen from an anther to a stigma: bees, flies, beetles, etc.
Polysaccharides	: long chains of monosaccharides linked by glycosidic bonds.
Prime swarm	: the first swarm to leave the parent colony, usually with the old queen.



Propolis	: sap or resinous materials collected from trees or plants by bees and used to strengthen the comb, close up cracks, etc.; also called bee glue.
Proteolytic activity	: the breakdown of proteins into smaller polypeptides or amino acids.
Pupa	: the third stage in the development of the honey bee, during which the organs of the larva are replaced by those that will be used by an adult.
Putrefaction	: the process of decay or rotting in a body or other organic matter.
Queen	: a fully developed female bee, larger and longer than a worker bee.
Queen cage	: a small cage in which a queen and three or four worker bees may be confined for shipping and/or introduction into a colony.
Queen cage candy	: candy made by kneading powdered sugar with invert sugar syrup until it forms a stiff dough; used as food in queen cages.
Queen cell	: a special elongated cell, resembling a peanut shell, in which the queen is reared. It is usually an inch or more long, has an inside diameter of about 1/3 inch, and hangs down from the comb in a vertical position.
Queen clipping	: removing a portion of one or both front wings of a queen to prevent her from flying.
Queen cup	: a cup-shaped cell made of beeswax or plastic which hangs vertically in a hive and which may become a queen cell if an egg or larva is placed in it and bees add wax to it.
Queen excluder	: metal or plastic device with spaces that permit the passage of workers but restrict the movement of drones and queens to a specific part of the hive.
Queen substance	: pheromone material secreted from glands in the queen bee and transmitted throughout the colony by workers to alert other workers of the queen's presence.
Rabbet	: a narrow piece of folded metal fastened to the inside upper end of the hive body from which the frames are suspended.
Rendering wax	: the process of melting combs and cappings and removing refuse from the wax.
Replication	: process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules.
Resmethrin (SSP-1382)	: a synthetic pyrethroid insecticide used to kill diseased honeybee colonies.
Robbing	: stealing of nectar, or honey, by bees from other colonies.
Royal jelly	: a highly nutritious glandular secretion of young bees, used to feed the queen and young brood.
Sacbrood	: a brood disease of honey bees caused by a virus.



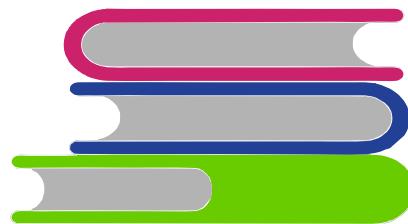
Scout bees	: worker bees searching for a new source of pollen, nectar, propolis, water, new home for a swarm of bees.
Secondary Metabolites	: a specialised metabolites, toxins, secondary products or natural products, are organic compounds produced by bacteria, fungi or plants which are not directly involved in the normal growth, development or reproduction of the organism.
Secondary swarm	: a smaller swarm which may occur after the primary swarm has occurred.
Skep	: a beehive made of twisted straw without movable frames.
Slatted rack	: a wooden rack that fits between the bottom board and hive body. Bees make better use of the lower brood chamber with increased brood rearing, less comb gnawing and less congestion at the front entrance.
Slumgum	: the refuse from melted comb and cappings after the wax has been rendered or removed.
Smoker	: a device in which burlap, wood shavings or other materials are slowly burned to produce smoke which is used to subdue bees.
Solar wax extractor	: a glass-covered insulated box used to melt wax from combs and cappings by the heat of the sun.
Spermatheca	: a special organ of the queen in which the sperm of the drone is stored.
Sporollenin	: the hard organic material that makes up the outer layer i.e exine of the pollen grain
Sting	: the modified ovipositor of a worker honey 13 cc used as a weapon of offense.
Streptococcus pluton	: bacterium that causes European foulbrood.
Sucrose	: principal sugar found in nectar.
Super	: any hive body used for the storage of surplus honey. Normally it is placed over or above the brood chamber.
Supersedure	: a natural replacement of an established queen by a daughter in the same hive. Shortly after the young queen commences to lay eggs, the old queen disappears.
Surplus honey	: honey removed from the hive which exceeds that needed by bees for their own use.
Swarm	: the aggregate of worker bees, drones and usually the old queen that leaves the parent colony to establish a new colony.
Swarming	: the natural method of propagation of the honey bee colony.
Swarm cell	: queen cells usually found on the bottom of the combs before swarming.



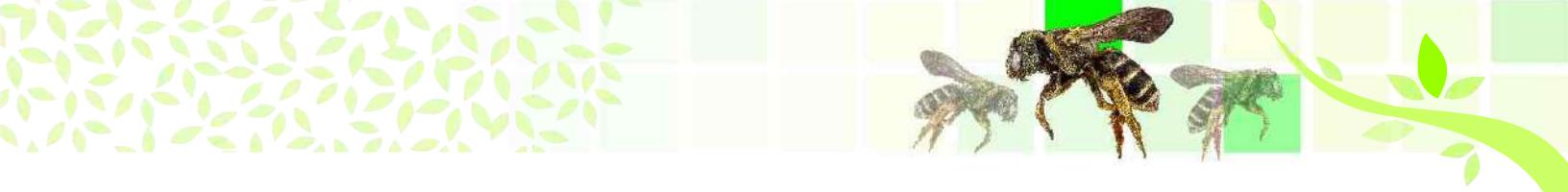
Tracheal mite (Acarapiswoodi)	: parasites that live in the trachea.
Terramycin	: an antibiotic used to prevent American and European foulbrood.
Therapeutic	: relating to the healing of disease.
Travel stain	: the dark discoloration on the surface of comb honey left on the hive forsome time, caused by bees tracking propolis over the surface.
Uncapping	: the process by which the thin layer of beeswax is removed, exposing honey frames for honey harvesting.
Uncapped Brood	: eggs and larvae not covered by wax.
Uncapping knife	: a knife used to shave or remove the cappings from combs of sealed honey prior to extraction; usually heated by steam or electricity.
Uniting	: combining of two or more colonies to form one larger colony.
Varroa mites (Varroa destructor)	: parasites that feed on the haemolymph of bees and reproduce on the pupae.
Virgin queen	: an unmated queen.
Viscin	: a clear, tasteless, sticky substance made from the sap of flowering plants. In some plants, pollen is held together in clumps or strings with viscin.
Wax glands	: the eight glands that secrete bees wax; located in pairs on the last fourvisible ventral abdominal segments.
Wax moth	: larvae of the moth <i>Galleria mellonclia</i> , which seriously damage brood and empty combs.
Winter cluster	: the arrangement of adult bees within the hive during winter.
Wired Foundation	: pressed wax foundation with wires embedded in the wax to add strength.
Wired Frames	: bee hive frames fitted with wires that helps to hold sheets of foundation in place.
Worker bee	: a female bee whose reproductive organs are undeveloped. Worker bees do all the work in the colony except for laying fertile eggs.
Worker comb	: comb measuring about five cells to the inch, in which workers are rearedand honey and pollen are stored.



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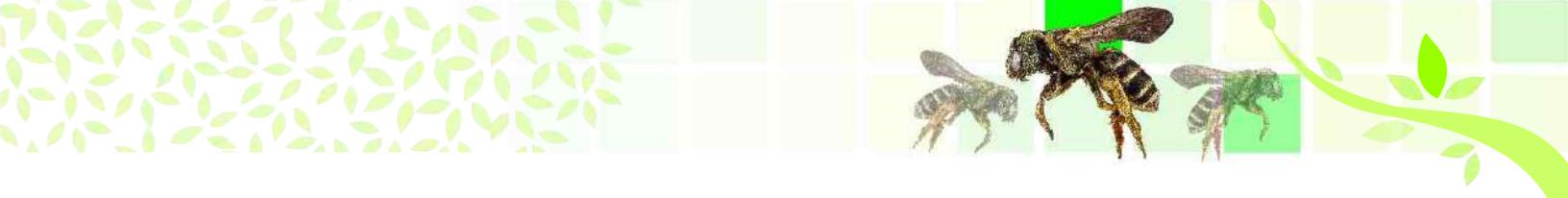
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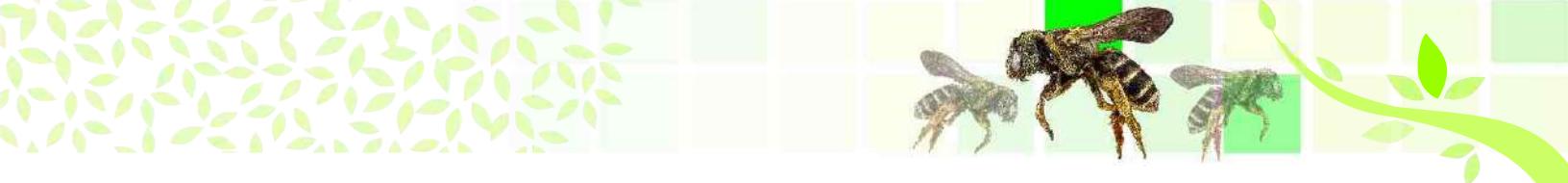
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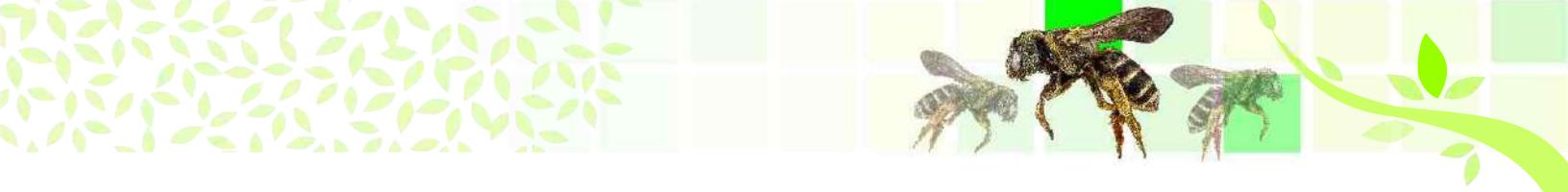
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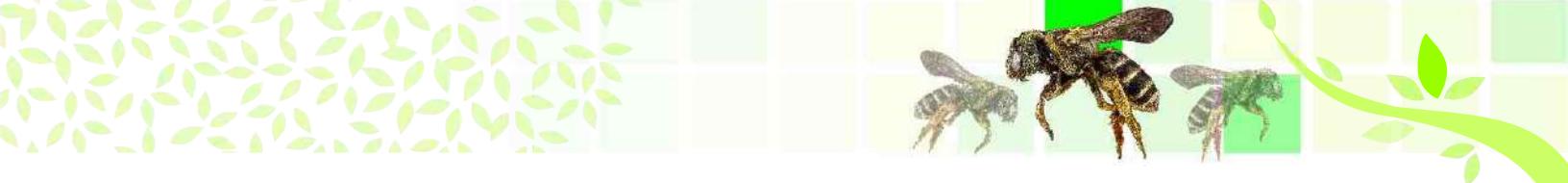
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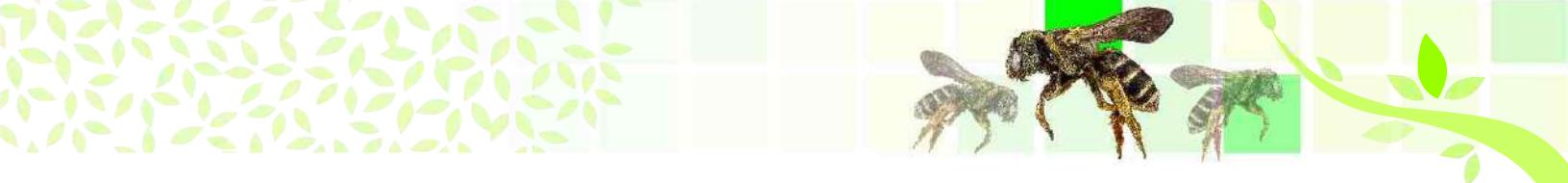
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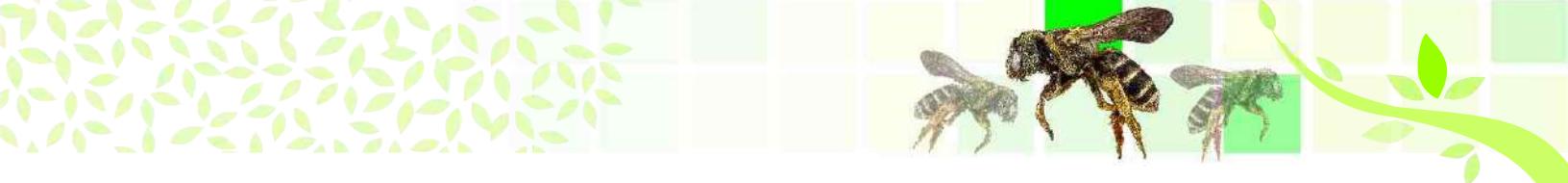
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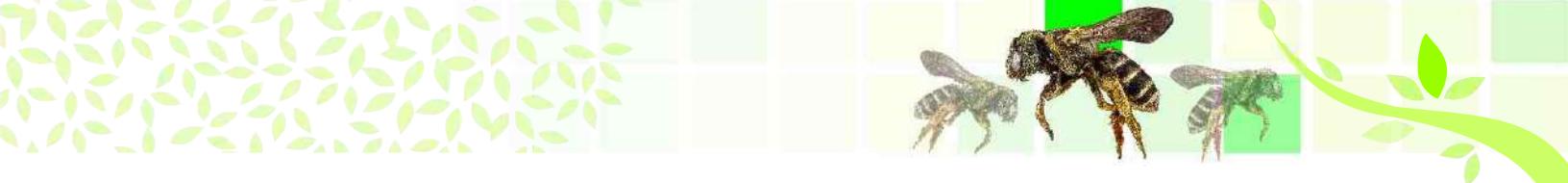
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