Epidemiology of Honey bee and the bacterial impact on honey bee population with reference to parasites of honey bee and their pathophysiology: Present, Past and Future

Abstract

In addition to being valued for its goods, the honeybee Apis mellifera is also valued for its role in pollinating both domestic and untamed plants. Arthropods, fungus, protozoa, bacteria, and/or viruses that are able to get past the individual and social immune systems of bees can infect the beehive. Infections are easily transmitted inside and between beehives because of the bees' close closeness to one another and their feeding behaviors. Additionally, the global spread of illnesses brought on by bee trading has led to a number of infections that significantly harm apiculture. Only rarely can an infection be identified by visual examination, by direct observation of the pathogen in some arthropods, or by pathogen-associated distinguishing characteristics. Significant progress has been made in the study of bee pathogens thanks to the development of molecular techniques based on the amplification and analysis of one or more genes or genomic segments. These techniques enable I the accurate and sensitive identification of the infectious agent, (ii) the analysis of co-infections, (iii) the description of novel species, (iv) associations between geno- and phenotypes, and (v) population structure studies. The genomes of bee pathogens have been sequenced, which has facilitated the discovery of novel molecular targets and the creation of specialised genotypification techniques.

Key Words: Genotypification, bee pathogens, *Apis mellifera*, bee viruses, Pathophysiology

Muhammad Zubair[1*], Arshia[1], Saira Boota[1], Khadija-tul-Tahira[1], Rimsha Maham[1], Maham Aslam[1], Aqsa[1], Anam Akhtar[1], Hira Malik[1], Afifa Maryam[1], Mubarak Ali[1], Umar Sajjad[1], Sabiha Aslam[1], Tahara Ashraf[1], Tayyaba Razzaq[1], Shabana Khadim[1]

Muhammad Zubair M.Phil Zoology (University of Veterinary and Animal Sciences Lahore Punjab Pakistan) (zubairmmzubair26@gmail.com)

Arshia under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (arshiachaudhryarshia@gmail.com)

Saira Boota under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (Sairamalhi3@gmail.com)

Khadija tul Tahira under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (khadiitultahira@gmail.com)

Rimsha Maham under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (rimshamaham42@gmail.com)

Maham Aslam under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (mahammirza43488@gmail.com)

Aqsa under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (sa6586777@gmail.com)

Anam Akhtar under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (anum11135@gmail.com)

Hira Malik under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (af6345846@gmail.com)

Afifa Maryam under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (afifamaryam0034@gmail.com)

Mubarak Ali under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (03425997522gsvfjf@gmail.com)

Umar Sajjad under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (Umar.sajjad98123@gmail.com)

Sabiha Aslam under Graduate BS Zoology (Aspire group of colleges Narowal, Narowal Punjab Pakistan) (basabohi@gmail.com)

Tahara Ashraf M.Phil Zoology (Government College Women University Sialkot Punjab Pakistan). (talatashrafmehmood@gmail.com)

Tayyaba Razzaq Graduated BS Zoology (University of Narowal, Narowal Punjab Pakistan) (taibarazzaq90@gmail.com)

Shabana Khadim Graduated BS Zoology (University of Narowal, Narowal Punjab Pakistan) (shabanakhadim70@gmail.com)

Corresponding Authors

Muhammad Zubair (+92 304-9521781) (zubairmmzubair26@gmail.com)

Saira Boota (Sairamalhi3@gmail.com)

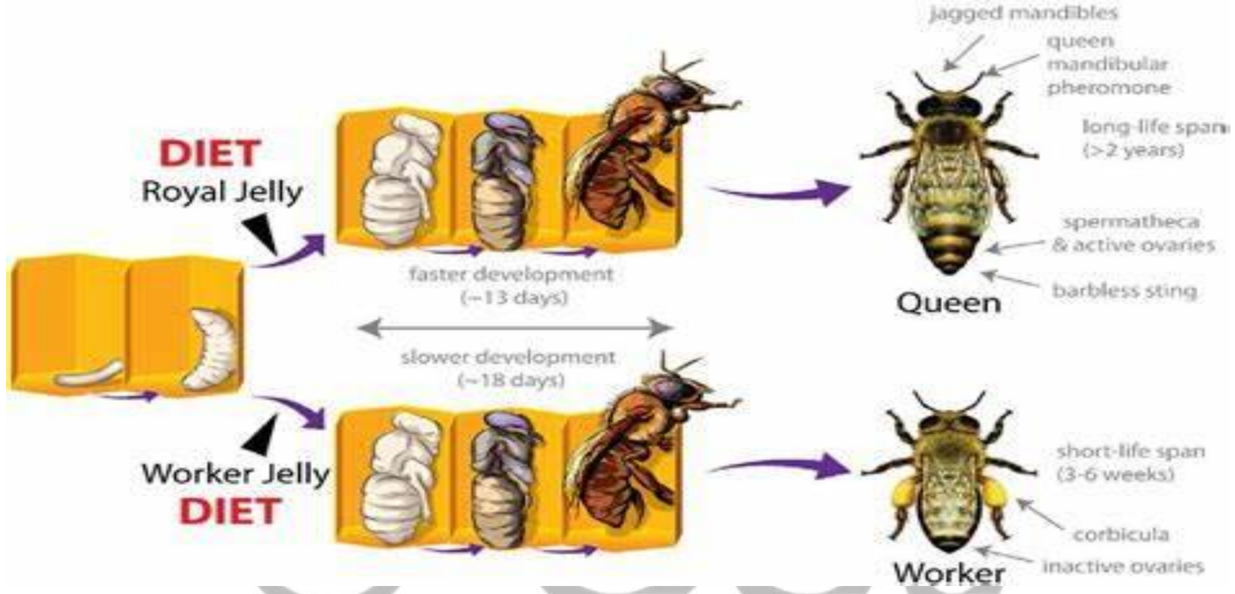
Mubarak Ali (03425997522gsvfjf@gmail.com)

1: INTRODUCTION:

The western species of honeybee, the *apis mellifera*, is very important to human society both economically and culturally. Bees help pollinate crops, which is a manner in which they help maintain the supply of food worldwide, but they also provide source of income for farms and beekeepers is associated with important cultural social ideas. In addition, nectar is an organic item with a variety of possible health advantages, and its exports and marketing are important sources of income for many countries across the world. A wide range of challenges are becoming more prevalent for managed honeybee colonies. One of these is their potential susceptibility to several illnesses caused by any one of the subsequent organismal classes: arthropods, viruses, protozoa, fungus, bacteria (Nagaraja *et al.* 2019).

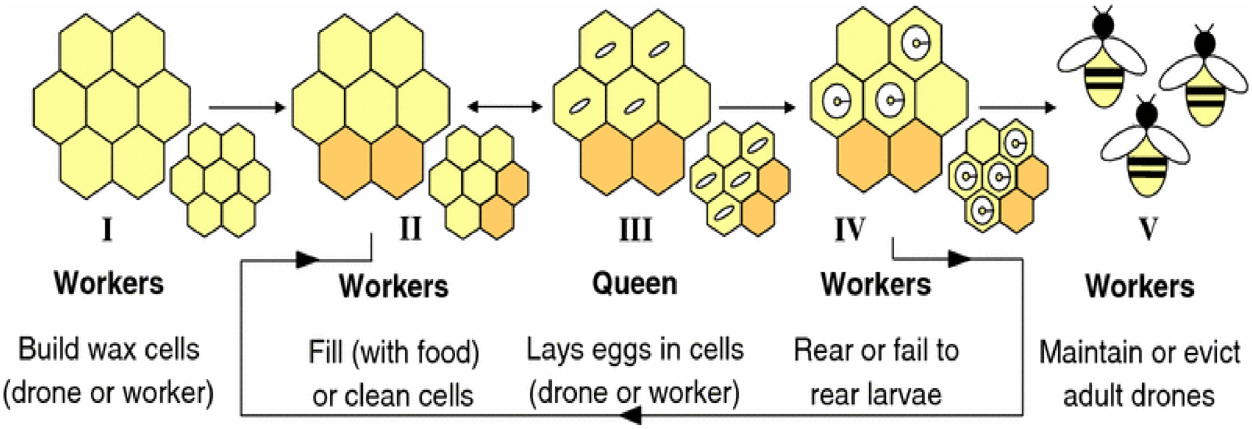
A honeybee colony is characterized by unique bee groups (drones, employees and queens) and stages of development (e.g., eggs, larval stages, pupae, or grown-up bees), every one of which serves as a possible infection target, in addition to the abundance of different environmental niches that are appealing to pathogenic microbes, such as humidity and warmth. Finally, honey bee foraging behavior provides a risk of pathogen transfer to and from other pollinators while gathering pollen and nectar. Notably, the beehive frequently experiences multiple infections caused by pathogen at once, seriously compromising colonies, health and making it vulnerable to other threats. According to humans play a significant part in supporting and fostering the transmission of honeybee illnesses in this environment (Traynor *et al.* 2020).

Because of the high density at which colonies are managed, disease is more likely to move across colonies and among natural and controlled workers. The rapid worldwide movement of honey bees, their reproductive systems, including their queens and sperm, as well as beekeeping supplies like pollen and honey, fosters the spread of illnesses, a few of them have become fully established on a worldwide basis. This is the case with the bee _infesting arthropod Varro destructor and its fungus microsporidium it coronae. Bees can defend oneself from diseases in their native habitat thanks to a variety of complex innate and social immunological mechanisms, however they are not adoptable to dealing with these exotic pathogens (Paris *et al.* 2018).



In order to overcome these challenges, more and more effective genetic tests are being developed in recent years. These methods make it possible to recognize bee diseases and make it easier to distinguish between various species and genotype with high specificity and sensitivity. There have been instances where the recognition of previously unknown organisms required the use of molecular technologies. For instances, the bee infecting protozoon crithidia mellificae was found to be representative of two distinct species, one of whom was given the name Lomaria and it is possible to distinguish V. destructor from V.jacobini. Additionally, molecular techniques have been developed to discriminate distinct species, like the microsporidia N. APIs and N ceranae that frequently confect humans. Notably, according to genetic evolutionary study, it was just recently suggested that both Noemi spp. Be reclassified as Vairimorpha spp. Along with to the well-known DNA amplification methods of PCR and its variations (nested and semi nested PCR, multiplex PCR, QPCR, and PCR_RFLP), loop facilitated isothermal amplification (LAMP) tests have been created for the identification of a variety of bee illnesses (Paris *et al.* 2018).

The former offers several benefits of ease of use, inexpensiveness, quickness, and absence of costly instruments like thermo cyclers or chromatography devices because processes happen during isothermal circumstances and result can occasionally be viewed with only naked eye. When a variety of DNA detection techniques are accessible, the approach to testing should be determined by the precise goals of the intended investigation, the nature of infectious agent and the specimen type, the available resources in the lab and the budget, as well as the inherent characteristics of each technique (Simone-Finstrom. 2017).(Liu *et al.* 2019)



Thanks to DNA sequencing, the variety and social structure of numerous infections have been identified. As a result, we can roughly determine when and where certain insects and fungal infection species, such as V. destructor and N ceranae, which are currently present around the world and pose a serious threat to beekeepers, first appeared. Recent advancements in cellular entering methods for certain pathogenic bacteria, such as multilocus order typing (MLST) and associated techniques, enable worldwide monitoring of varieties along with the ability to identify the source and their way of epidemic transmission (e.g.; paenibacillus larvae by stable wgMLST, melissococus plutonium by MLST) (Ngor *et al.* 2020).

2: DESCRIPTION OF MAIN BEE PATHOGENS AND MOLECULAR METHODS FOR THEIR DETECTION

2.1: Brief overview

Few microbes can infect honeybees and lead to illnesses. Only honeybees have been discovered to be affected by the most common and renowned infection caused by Melissococus plutonium and paenibacillus larvae. Two more important but occasionally disregarded and possibly undervalued bacterial illnesses are Spiro plasma melliferum and S. apis, which have been reported to affect either honeybees and bumble bees as well. Furthermore, the fungus serration marc scene has been isolated from a wide range of sources that include over 70 various species of insects, plants, small mammals and in certain instances, treated human patients (Bird *et al.* 2021).

2.2: Paenibacillus larvae, the causative agent of American Foulbrood:

Paenibacillus larvae, a widely dispersed, gram positive, spore producing bacterium of the formicates as phylum, is responsible for American Foulbrood (AFB), which is among the most pervasive and harmful diseases to affect honeybees. Spores are fed to larva by nursing bees so they are able to penetrate the lumen of the intestine with spore contaminated food. The bacteria advances to the photosynthetic microbial stage, infiltrate, and proliferate in the hemocoel of insect larvae producing eggs if infection happens within the first few hours of exposure. Millions of spores are produced by each infected larva, and only eight spores are required to initiate another outbreak (Ribani *et al.* 2020).

Infections can be directly transmitted to young larvae by nurse bees or by spore that remain at the bottom of a brooding cell. The virus can move to other colonies when combs are exchanged, tainted honey is utilized, or sick queens are moved. Spores are extremely heat and chemical resistant and can last for years. Antibiotics are also capable of eliminating bacterial plants; however, they are ineffective towards spores. As consequently, burning down infected hives and apparatus is one of the few options available to limit the propagation of AFB. Because of an arrangement of normally closed brood, unsealed cells with unhealthy larvae remain, and empty cells, the combs with severe AFB infections appear mottled. These clinical signs, according to estimates, begin to show up with an average of 3000 spores each bee the matchstick test is frequently used to confirm sickness suspicions. It entails piercing the corpses of dying larvae and peering inside to see whether or not there is a brown, slippery, ropy string inside. However, false negative results might occur when the larval remnants are moist (Matthijs *et al.* 2020).

A number of detection methods, including community cultivating and evaluation, biological assessment, antibody-based methods, and microscopy, are effective for detecting AFB early on before the onset of medical signs, but they also allow for the verification of P. larvae infection shortly after the beginning of disease. The methods are not further examined in this review because they are adequately described elsewhere and function best in labs lacking molecular biology equipment. Methods for P. Larvae_ specific DNA amplification can be utilized with spore /cell solution samples after cultivation or with diseased larvae, wax, honey, pollen, or beehive debris directly. When working with cultural bacterial suspensions, a fast-warming phase to 95 degrees Celsius is all that is necessary for determining the species by PCR, even though there are readily accessible kits for DNA extraction. It has been noted that treatment with an enzyme known as EDTA, and Triton X_ 100 can improve recovery of DNA from honey sample prior to the release of packaged DNA extraction kits. Two alternative genotyping methods with greater accuracy and higher precision of P. larvae the genotypes are being stated; two multi locus order writing (MLST) systems, all constructed using an independent set of seven housekeeping genes that are capable of distinguishing 15 and 21 sequence types (ST), correspondingly; and a multiple_ locus parameter amount of conjunction continue evaluation (MLVA) method that differentiates between at least 23 different VNTR types (Liu *et al.* 2020).

2.3: Melissococus Plutonium, causing European Foulbrood:

Melissococus plutonium causes European foulbrood (EFB), a fatal disease that exists across every continent in the world. M. plutonium is an anaerobic or microaerophilic, gram positive, non-spore forming bacteria belonging to the phylum Formicates and family Enterococcacae. Streptococcus pluton, which was discovered in 1912, was afterwards red scribed as M. plutonium infection occur in larvae who eat contaminated worker bee food, and the bacterium's development in the larvae's stomachs result in nutrient deficiencies (Gómez-Moracho *et al.* 2020).

Furthermore, the infectiousness and lethality of this bacterial disease seems to be associated with a plasmid that carries the poisonous substances melissotoxin A, which is likely damaging the intestinal cells. EFB is frequently misunderstood to be a mild form of AFB, despite the fact that the two illnesses are brought on by different viruses. The OIE handbook of tropical animals contains information about EFB. Both times, the larvae have problems, death is greater when it occurs soon after hatching, and the combs have blotchy patterns. AFB and EFB are distinct from each other in that afflicted hives typically contain more closed or unbound brood, correspondingly (Williams *et al.* 2019).

Due to the difficulties associated in the in vitro production of M. plutonium and the reality is that cultivated populations are no virulent until association with other kinds of bacteria occurs, molecular diagnostics is advised over cultivation in vitro for the early detection of EFB. Since the entire genome of M. plutonium was discovered in 2011, it has sped up the advancement of genetic testing and made it possible to learn more about the variations between strains and the genetic epidemiology of this bacteria. The selected gene for molecular identification by simple, semi nested, or qPCR has primarily been the 16S rRNA gene. Similar to P. larvae, M. plutonium genomic DNA may be extracted from specimens of adult and larval bee phases as well as pollen and hive detritus (Meixner *et al.* 2014).

One of the extra gene targets used was the melissotoxin A gene expressed by the plasmid. The Soda (manganese dependent superoxide dismutase) gene for qPCR. Along with to the PCR based diagnostic listed above, the following two additional M. plutonius DNA detection procedures are especially useful for the examination of a lot of samples: a colorimetric nanoparticle-based detection approach and a LAMP protocol that targets the gyrb (DNA gyrate subunit B) gene are both used. The first method has advantage such as a 10 times higher detection limit than a semi nested PCR based on the 16S rRNA gene and it doesn't need specialized tools to analyze the data, whereas the second method is set apart by its rapidity and low cost (Simone-Finstrom *et al.* 2012).

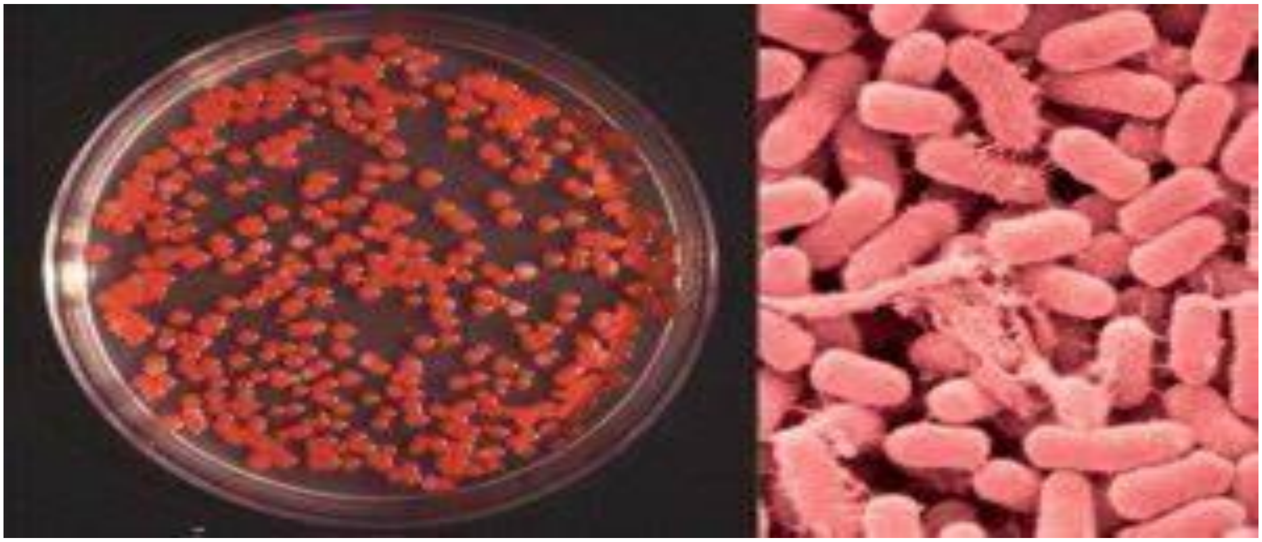
2.4: Spiro plasma Apis and S melliferum causative Agents of May Disease:

Spiro plasma apis and S. melliferum are two examples of the gram-positive bacteria that make up the Molecules class of the tenericutes phylum. Both have helicoidally forms, no cell walls, and are typically motile; however, isolated infections have frequently observed. All affect the blood cells of bees. The infection causing process of these microbes in honey bees are not well understood, but it is likely that they involve their capacity to migrate through the gastrointestinal tract epithelium to the hemocoel, affect and divide within the cell in tissues, and divide outside the cell in the hem lymph, which causes movement decline and death (Kojima *et al.* 2011).

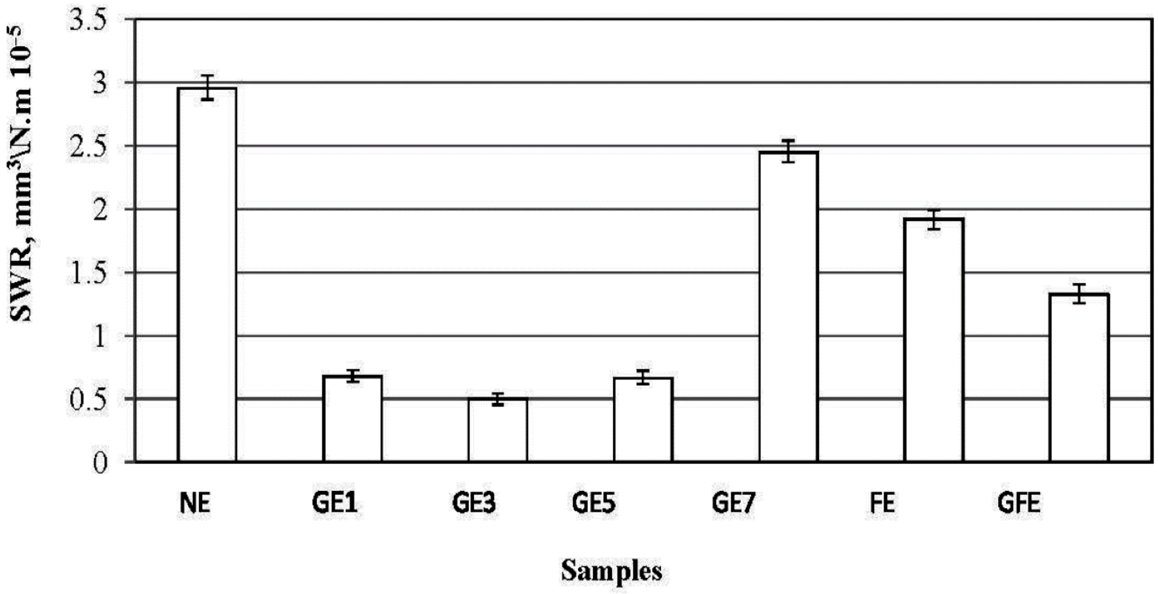
Despite the latter’s apparent wider geographic spread, experimental study has revealed that S. melliferum infections. Spiro plasma spp. has been suggested as a potential cause of May illness, a neurological ailment that strikes European bees in the springtime and is connected to flowering peaks. While these microbes are present in nectar, contact with flowers might cause bees to become ill. Similarly, it has been shown that bee infections with S. melliferum in the USA are seasonal, peaking in May. Examples of molecular research include the creation of molecular detection methods and the genome sequencing of these infections. Table 1. Species specific primers were used to ramp up the S. melliferum spiralin and S. APIs rob genes, which were then combined with common primers against Spiroplasma sp. To enable the detection of both species after isolation of each bacterium. In contrast, a qPCR assay using S. melliferum spiralin and S. apis 16S rRNA primers allowed for highly accurate species-specific detection (Ribani *et al.* 2020).

2.5: Serratia marcescens Causing Honeybee Sepsis:

A gram-negative bacterium called serratia marcescens can cause sepsis in both plants and animals. It is a member of the order Enterobacterales and phylum protobacteria. Studies on S. marcescens have mostly focused on its importance to the wellbeing of humans, particularly the characterization of diverse strains and the complete sequencing of its genome. Even though A. mellifera has been the subject of sepsis for more than a century, recent years have shown substantial advancements in our understanding of S. marcescens pathogenicity in this insect. Studies have shown that this bacterium is frequently found in the microbiota of bees and act as a pathogenic opportunistic organism with the potential to be lethal in the correct situations (Matthijs *et al.* 2020)



There have been a few studies on molecular recognition of S Marcescens' in bees that relay on sequencing 16S rRNA gene sections replicated by a polymerase chain reaction, or PCR, utilizing general primers, which are inadequate for identifying specific species. Therefore, the establishment of genotyping and genetic testing for rapid and accurate detection would aid in understanding of this disease by researchers. Importantly, the 16S rRNA amplicons analysis by denatured gradients electrophoresis with gel (DGGE) allowed for the detection of a putative new S. marcescens strains. Spiro plasma spp. and S. marcescens have been found frequently in studies of the bee pests including V. destructor and AFB; this finding supports both of these organisms are opportunistic infections (Liu *et al.* 2020)



A few findings on the molecular identification of S. marcescens in bees rely on sequenced 16S rRNA gene sections amplified by polymerase chain reaction (PCR) using universal primers, which are insufficient for detecting individual species. As a result, the development of molecular diagnostics for precise and quick detection, as well as genotyping, will help researchers better understand this disease. Importantly, the identification of a potential new S. marcescens strain was made possible by the 16S rRNA amplicons analysis by denaturing gradient gel electrophoresis (DGGE). Notably, investigations of the bee micro biome and of bee pests like V. destructor and AFB have commonly detected Spiroplasma spp. and S. marcescens with significant frequency; this finding confirms that both of these species are opportunistic infections (Alaux *et al.* 2014)

Table1: Molecular assays for the detection, interspecific differentiation and intraspecific genotyping of bacterial pathogens of the honeybee.

Reaction Type	**Genotype**	**Goal**	**Amplicons type and size**	**Primer**	**Reference**
Spiroplasma apis and *S. melliferum*					
multiplex qPCR	*S. melliferum*	*spiralin*	160	M59366	(Grozinger *et al.* 2015)
	S. apis	16S rRNA-ITS1	190	AY736030	
	A. mellifera	RPS5 gene	115	GB11132	
P. larvae and *M. plutonius*					
multiplex PCR	*M. plutonius*		281	AY862507,	(Paris *et al.* 2020)

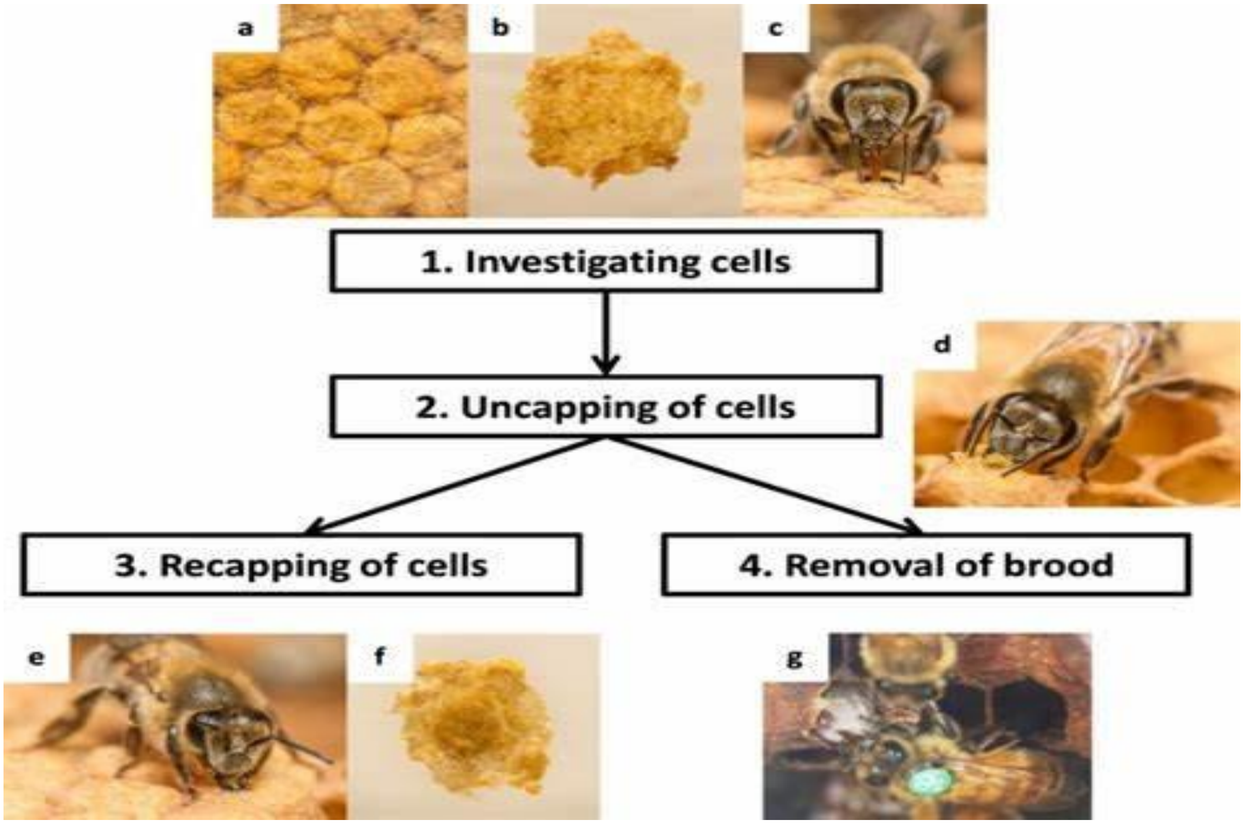
	Ascosphaera apis	16S rRNA	136	U68313	
	P. larvae		973		
multiplex qPCR	*A. mellifera*	*tnp60*	87	AB023025	
	P. larvae	*actin*	95	CP003355	
Melissococcus plutonius					
MLST	STs cluster into clonal complexes CC3, 13 and CC12	*argE*, *galK*, *gbpB*, *purR*	ST1-27; ST1, 4,8,9,13,14, 15,17,18,20,26: CC13;	HF569117-42	(Ramasamy *et al.* 2017)
	qPCR	16S rRNA	67	EF666055	
	typical strains (CC3, CC13)	*Fur*	45	AB778538	
	PCR	melissotoxin A	23	X75752	
	16S rRNA	16S rRNA	56	KMT29105	
Paenibacillus larvae					
PCR	ERIC I + II	metalloproteinase	33	AF111421	(Shende *et al.* 2021)
			76		
			23		
		16S rRNA	46	AY030079	
			38		
			28		

3: ANTHROPOGENIC DIRECT DRIVERS ASSOCIATED WITH HONEY BEE COLONY DECLINE:

Variables referred as anthropogenic variables, as well as various illness and pests known as direct biological travelers, cause colonies deaths. Combining all among those variables frequently results to illness, death, including colonies failures. During this analysis, we'll concentrate upon those components individuals believe to be the most vital (Siddique *et al.* 2020)

3.1. Pesticides:

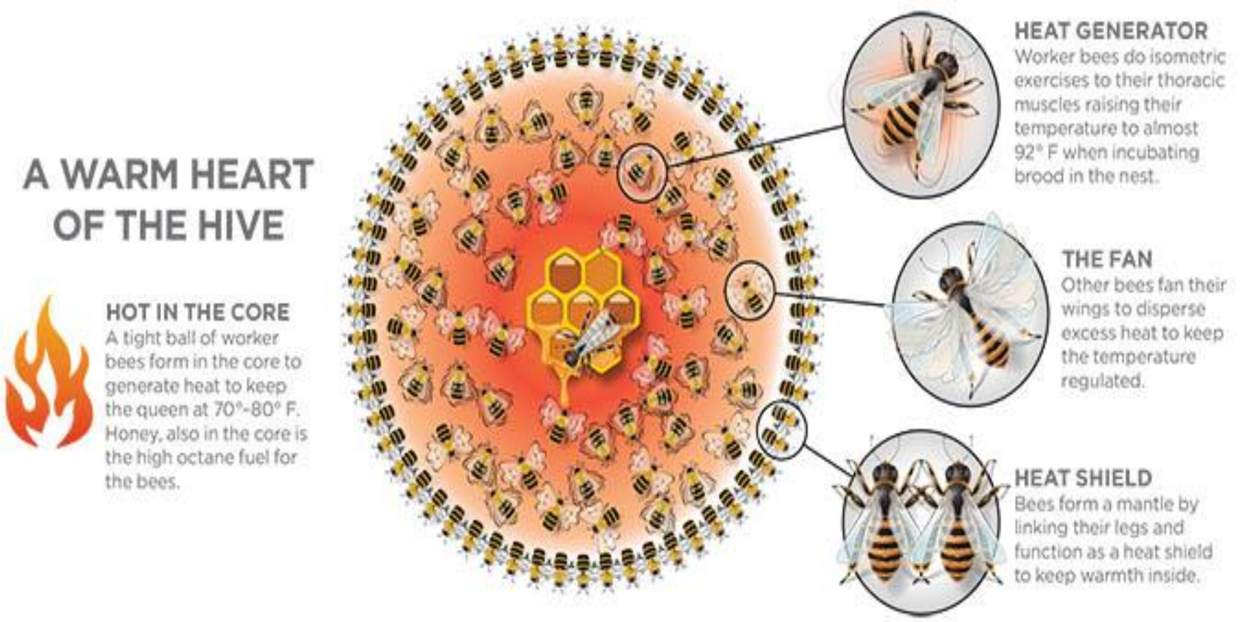
The apiarist are now beginning to utilize agricultural pesticides to fight parasitic insects in woodland as well as various ecological system along with numerous crops. Pesticides are hazardous substances having a particular mechanism of action, which frequently impacts a specific metabolism in a unwanted species. Pesticides are applied to the crop through a number of crops, like aerosols germ finishes etc. bees are subjected to pesticide through both nectar and pollen of damaged plants who carry residues of the chemicals (weeds or crop plants around the grounds). The greatest risk to bees is from insecticides (Simone-Finstrom. 2017)



That's why beekeepers use preventing measures when utilizing them close to their hives or bee colonies. A lot of countries give information to beekeepers to reduce the poisonous effect of bees when they work on different crops. These preventing measures are not used by whole word or countries, which cause the death of bees or the extinction of whole apiaries. Neonicotinoids, a new class of insecticides have a received a lot of attention recently from both the academic and beekeeping sector. They are commonly used for therapeutic goods, chemicals for managing insect pest in fishery culture and protection of plants. Neonicotinoids show a neurotoxic substances and their effect is more significant on insects than on mammals. Their neurotoxic effects manifest as a chemical amplifier on the synaptic membrane neuronal acetylcholine receptors which are involved in a variety of intellectual functions (Sulaiman *et al.* 2020)

3.2. Climate change:

The timing of insect fly and the blossoming of plants may change as a result of environmental change. Insect deaths brought on by phenological abnormalities impair the pollination of plant in a negative way. Climate play a significant role in regulating humidity and temperature. The brood temperature should be kept at 34 degree Celsius, the level of moisture in colonies should be kept as minimal as feasible, and in winter time, the temperature of hives core cannot fall under 13 degree Celsius. This is crucial, and to keep such temperature and live, colonies of honey bee need to have adequate availability of the carbohydrates (Tejeda-Serrano *et al.* 2017)



Prolonged times during icy or rainy weather, as well as a shortage of nutritional sources can also negatively affect the fitness of honey bee colonies. These conditions can restrict and fly movement and reduce the amount of pollen and nectar available inside the hive. Compared to low temperature, brood temperature beyond 34.5 Celsius cause behavioral change in bees, as well as problems with memory and learning. According to a survey of honeybee colonies declines in the USA, the weather impact on colonies of bee are a significant contributor to CCD. CCD is associated with starvation as well as shifts in bee's ecosystem and both of which indirectly brought on by environment factors. In addition, environmental factor could enable introduced species to take over honey bee hives, which will further harm population of honey bees (Tripathy *et al.* 2021)

3.3. Environmental pollution:

Honey bee engage in environmental interaction, particularly when gathering nectar and pollen for food. Animals consequently appear regular interaction with certain compounds and environmental pollutants. In the natural world, plants take in and preserve pollution and harmful compound, which are typically industrial pollutants, automobile exhaust pollutant, chemical pesticide and insecticides. Anthropogenic elements are the main reason of air pollution, (mobile sources, urbanization, energy generation, industrialization and other pollutants).

Contamination with heavy metal ranks as one of the most significant effect of air pollution. It is well recognized that’s a number of oxidation, including zinc ,copper, cobalt, nickel, cadmium, mercury and lead, negatively impact both nectar (directly) and the honey bees that consume it. (Indirectly). Since bees gather pollen from a variety of plant, toxic metal are frequently found in high concentration within plants that are damaged, which increase the quantity of harmful metal in bees bodies and poisons them. Because of this honeybees and product made from them are employed as biological marker of heavy metals contamination in the environment (Unuofin *et al.* 2020)

3.4. Bee management:

Certain features of beekeeping may be the primary cause of colony collapse or may serve as a food product for the complexity of stresses that might cause it. They involve the excessive harvesting of bee product, artificial means, unidirectional consuming food, the use of medicines, acaricide, pesticides in hives, being exposed to unfavorable temperatures and alterations in temperature, illnesses and parasites, and uncertain supply of drones and queen.one sided choice among honey bee causes the population of species to experience genetic degradation and a shortage of immunity to infectious illnesses, the mites, beekeeping acaricide used in beehives etc (Vyas *et al.* 2019).

3.5. GMO crops:

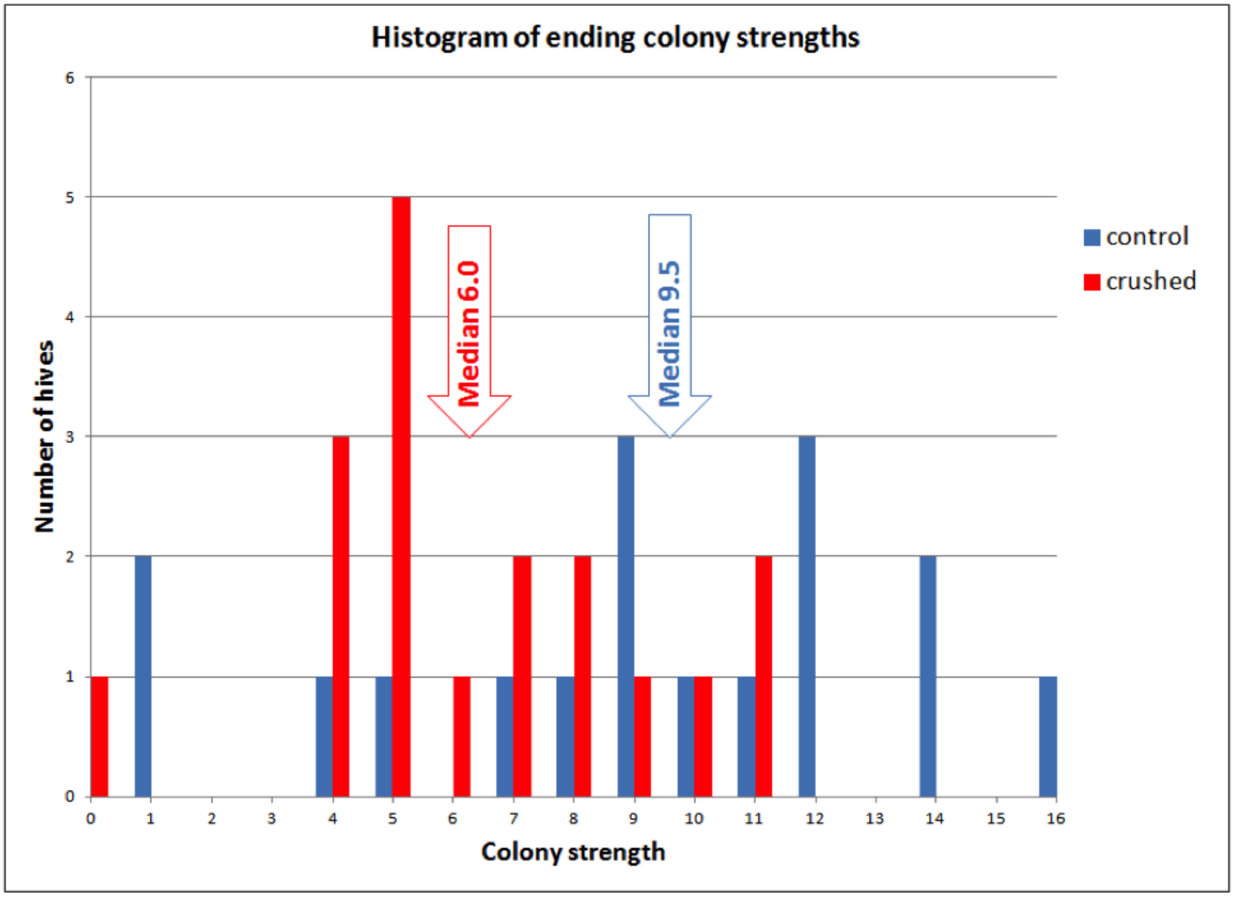
In 1996, genetically modified maize and varieties of soybeans with genetics for tolerance to herbicides and pesticide production were first released in the USA. 113 million km of GM crops were planted in 2007 across the globe, despite the EU being one of the outliers. Honeybees and other insecticide protection has become a source of worry as the region being cultivated using these crops has grown. Numerous researches have been carried out with multiple species of plants that have Bacillus thuringiensis BT genes for immunity against insect pests. Insect _resistant (IR) crops, such as those that produce Bacillus thuringiensis (BT) toxins, have not been found to have any direct lethal impact on the honey bees or other Hymenoptera, although certain sub lethal impacts on honeybee’s behavior have been found. GMOs have conflicting effects upon the wellness of honey bees. Although some investigators did not see any variation in honey bee behavior or learning, some have shown that consumption of high quantities of BT toxins impacted honey bee behavior. Lesser toxin amounts, like those seen in other engineered types, had no impact. Toxins found in GMOs crops result in decreased the survival of larvae and body bulk as well as longer honey bee developing times. On the other side, the development of cultivars resistant to specific herbicides and the development of clear field technologies have made it possible to produce crops like sunflower, maize, etc. without being overrun by weeds. The extensive use of technology is said to have been a few of the factors leading to the starving of bees both natural and cultivated since weeds are an alternate source of forage (Williams *et al.* 2019)

4: INTERACTIONS BETWEEN DIFFERENT DRIVERS:

There is ample evidence that certain stressors may interact negatively or add to one another, harming the wellness and survival of honey bees in addition to the unique impacts that each pressure has on them. The following are the key interactions between drivers: 1.climate change and land use, for example, because of global climate change, a pollinator species may simply move to a new geographic area, increasing the wide range of pollinators in the recipient region. The competitions for food supplies or the spread of various parasites and diseases make this movement unpleasant for the native fauna in many circumstances. The impact of viruses and insecticides is another example of how drivers can work together synergistically. Several researchers have noted an increase in larval or worker honey bee mortality as a result of the additive or synergistic interactions between Nosema ceranae infection, sub-lethal dosages of neonicotinoids, and (BQCV) 3. in order to maintain optimum growth and reproductive BQ, honey bee colonies require a diet that is balanced and appropriate for bees, as well as one that is free of stress from disease and pesticides. Several direct anthropogenic factors can change variety and have the potential to wipe out many flowering plants, which are the main source of food for honey bee. There is a higher danger of the individual and combined impacts of pesticides and infections on honey bees as a result of these anthropogenic interventions, which could result in malnutrition and potentially reduce immune system activity and the function of several critical detoxification enzymes. It is evident from what has been mentioned so far that the interaction between anthropogenic direct drives may pose a significant threat to the health and survival of honey bees (Zhang *et al.* 2020)

4.1: Viral impacts

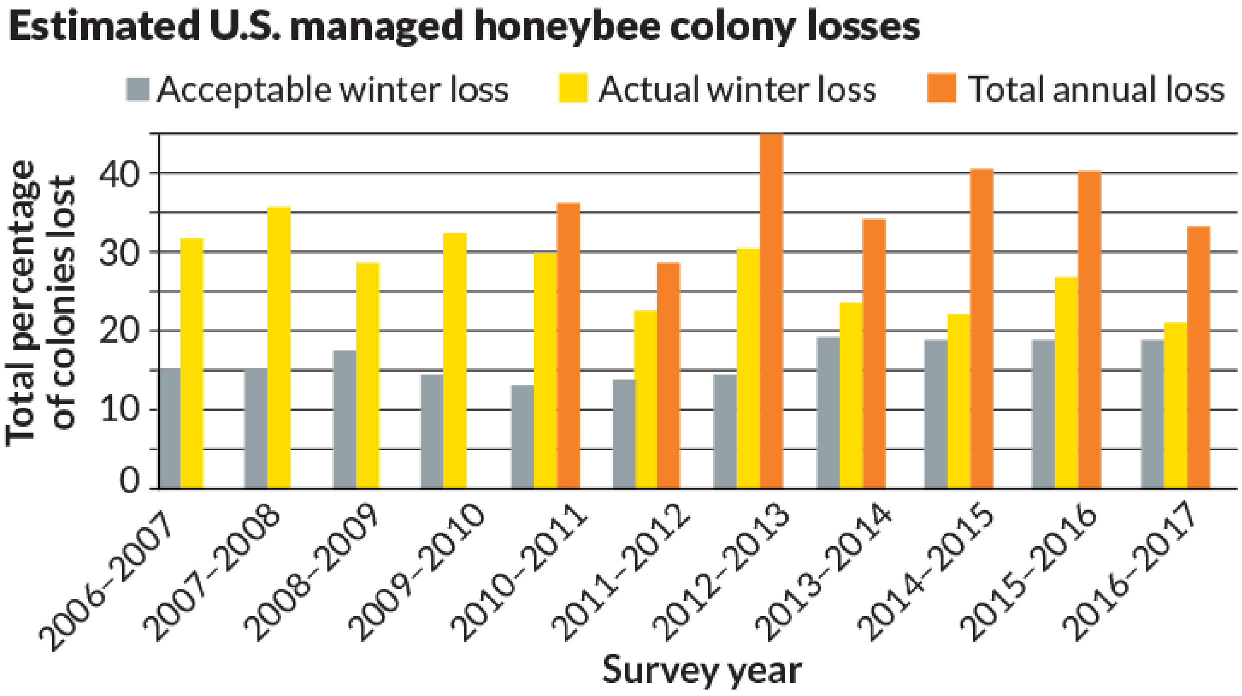
Honey bee colonies face a variety of diseases that are brought on by potential pathogens and altered environmental factors in different ways. Scientists found that annual colony losses were high in regions of the world responsible for the provision of food for all, so it is crucial to identify those people or organizations who are in charge of these problems. Many environmental issues, including as intensive agriculture, which frequently uses pesticides, a lack of essential food sources, habitat loss, diseases, and pests, all have a detrimental impact on bee fitness.



Moreover, infections pose serious risks to the health of honey bee colonies. Although similar viruses including ABPV, KBV, IAPV, DWV, and LSV2 are found in weak or CCD_afflicted hives, colony level research identifies these connections as being rare. Colony collapse disorder (CCD) is characterized by the rapid extinction of adult bees from the hive and the eventual queen's presence with few newly emerging bees. The resulting harm agriculture and apiculture worldwide was estimated to be worth around $75 billion. Research showed that IAPV was connected to because the risk of colony collapse is increased by its presence in honey bee colonies. Likewise, colonies exhibiting CCD symptoms also included KBV (Tong *et al.* 2022)

4.2. Declines of reared honey bee

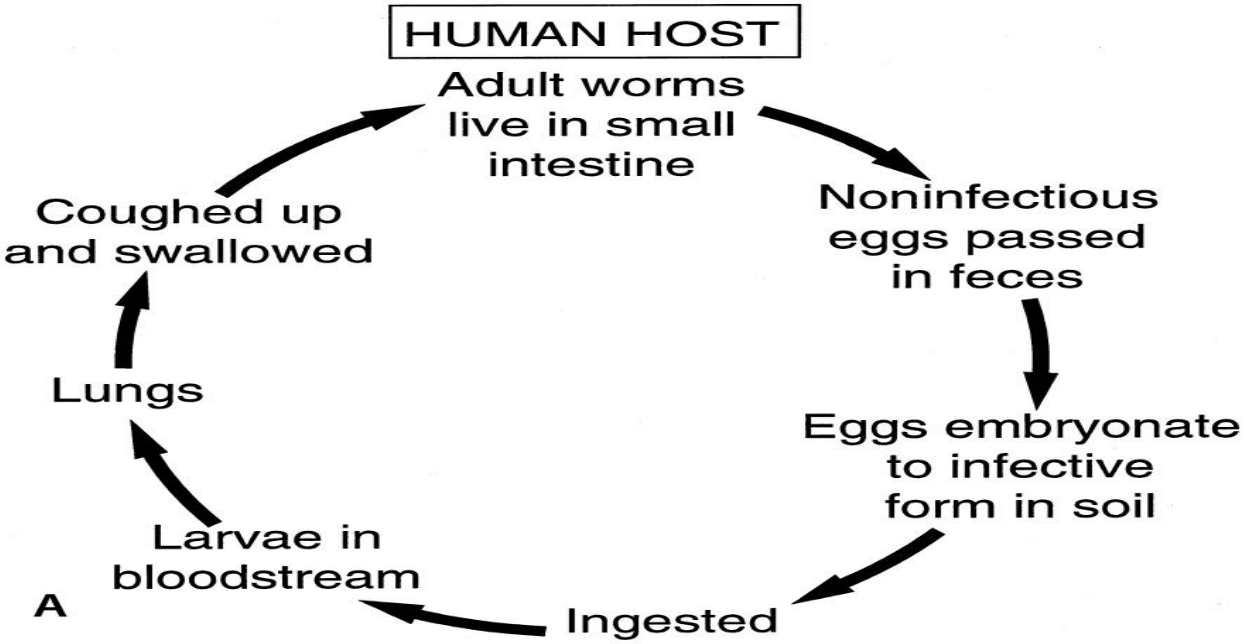
Controlled honey bee populations are experiencing a decrease as a result of many interrelated factors, such as usage of pesticides, parasites and disease pressure, and habitat destruction. According to reports, honey bee infections can spread to other kinds of honeybees as are primarily for the loss of those species. According to research, the number of honey bees raised in Europe is on the decline, with the number of colonies in central European countries consistently declining while marginally increasing in Mediterranean nations. In the United States and Europe, controlled honey bee populations frequently had BQCV.



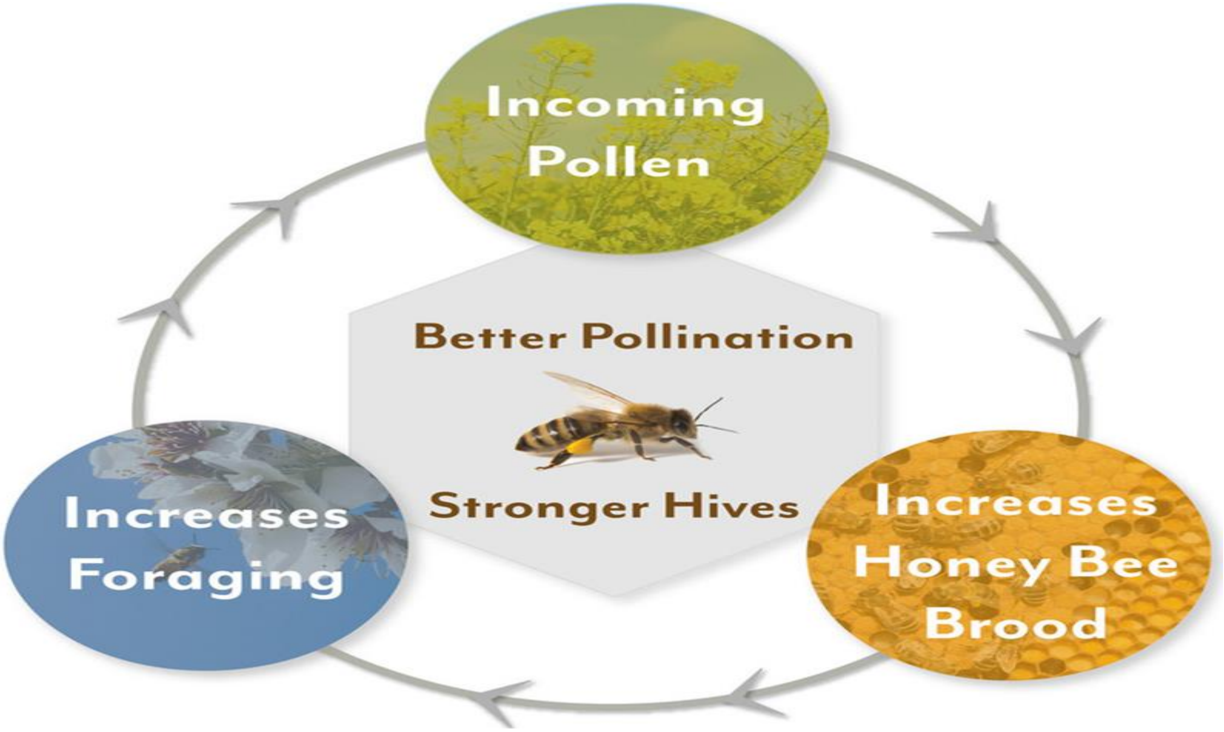
According to a research of 26 sites in England, honey bees were more likely to be infected with BQCV than other bee species. Similar to how IAPV was common in North American honey bee colonies but only sometimes seen in European apiaries. According to research, controlled bees and wild ones can both spread viruses to one other. Additionally, various kinds of bees can harbor and transmit viruses, and the severity of the infection relies on the host organisms and the type of virus as well as a few other characteristics, such as the age, gender, nutrition and genetic makeup of the host (Zhao *et al.* 2018)

4.3: The effect of viruses on honey bees under nutritional stress:

The effect of food quantity and quality on mammals contact with infections and stressful circumstances. This information about honey bee is also not widely understood. But both nectar and pollen have distinct components, including as lipids, carbohydrates, proteins, as well as additional phytonutrients and necessary elements (such as minerals and vitamins), which have a positive impact on the health of their immune systems. Additionally, different food sources have a significant impact on the health of bees. Additionally, pollen and nectar are essential for the continued survival of adult and larval bees, delivering essential nutrients. Poor nutrition, on the other hand, reduces the ability of bees to forage, which has an adverse effect on both the person and colony levels. As a result of obvious IAPV viruses and N, studies have demonstrated that a diversity of pollen have a significant impact on regulating the innate immune response and lowering death rates.



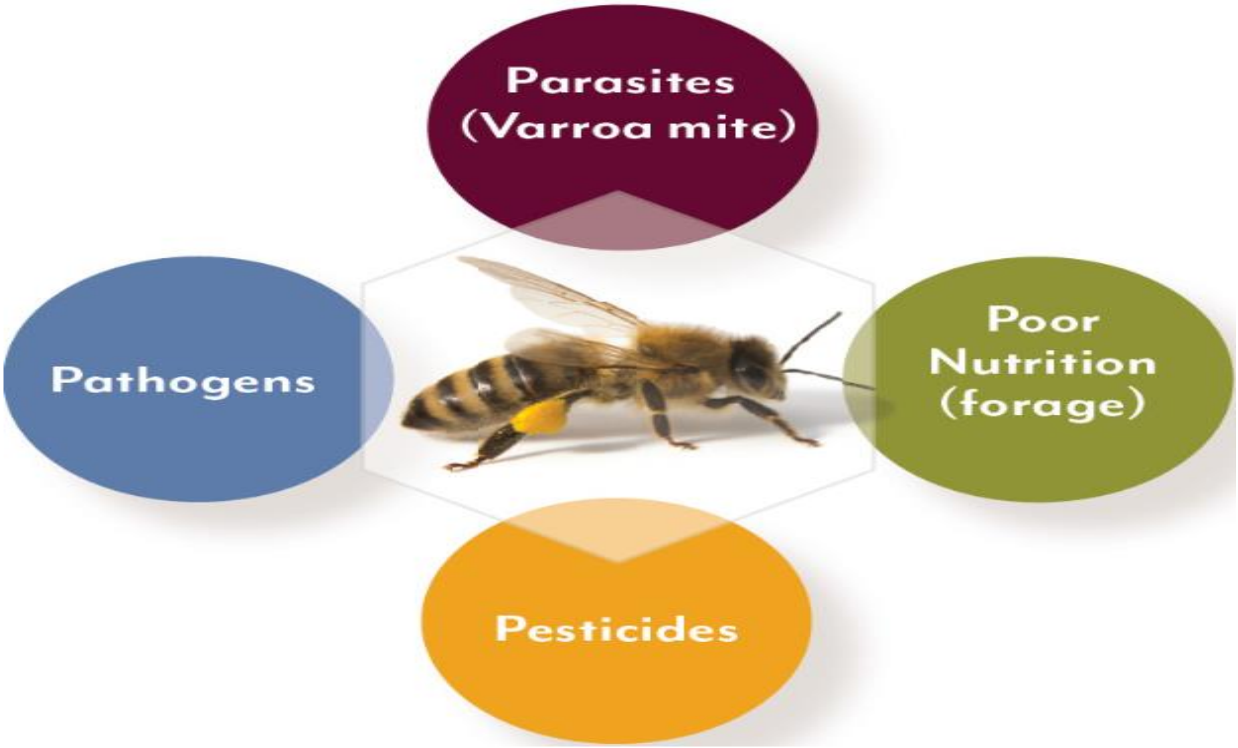
Because changes in land use practices result in a change in floral characteristics and have an adverse effect upon honeybee morphology and fitness, the ability of honey bees to supplies of food has become concerning. Additionally, hives near more developed areas suffer from greater colony losses and have lower fat assemblages in the winter. However, this is not always the case because sometimes increased crop growth leads to a reduction in pollen storage and honey output. However, research from the western United States found that agricultural areas produce more honey than urban areas do (Alaux *et al.* 2014)



Infections with N. apis and N. ceranae in honey bees disrupt digestion and affect the hosts immunity, which makes bees malnourished, immunosuppressed, and vulnerable to infections with other viral diseases including SBV and huge numbers of DWV particles. The spread of infections, such as SBV and Nosema species. Increase the hormone levels of young bees, which leads to early foraging behavior, and decrease the nutritional value of a honey bee colony by lowering pollen gathering and vitellogenin storage (Grozinger *et al.* 2015).

4.4. Effect of viruses on foraging performance of honey bees:

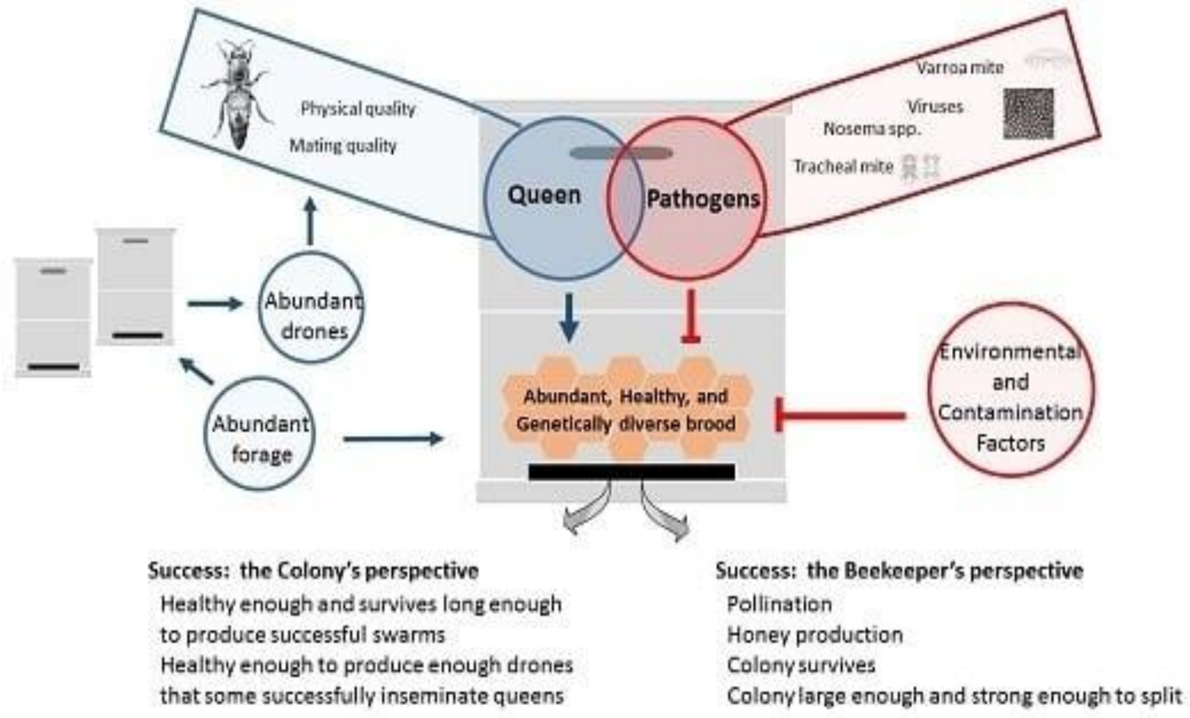
While honey bee are the primary regulated invertebrate pollinator in the globe wild bees are also an important component of ecological system and can increase the production of crops. A melliferum the honey bee that produces honey is an important character for agriculture since it pollinates several food crops which bring in over fifteen billion dollars yearly. Because honey bees are important and well known for their pollination tasks which provide food and bee’s populations are gradually declining throughout the world there is a lot of emphasis on bee preservation today. For example fruits, nuts, berries, seeds, leaves and roots. They not only pollinate crops used by humans but also by birds and other living things throughout all over the world. By feeding on honey bees Varro mites are able to spread highly contagious diseases through their host and induce a variety of changes including a reduction in weight lower immunity shortened lifespans and reduced exploring ability.



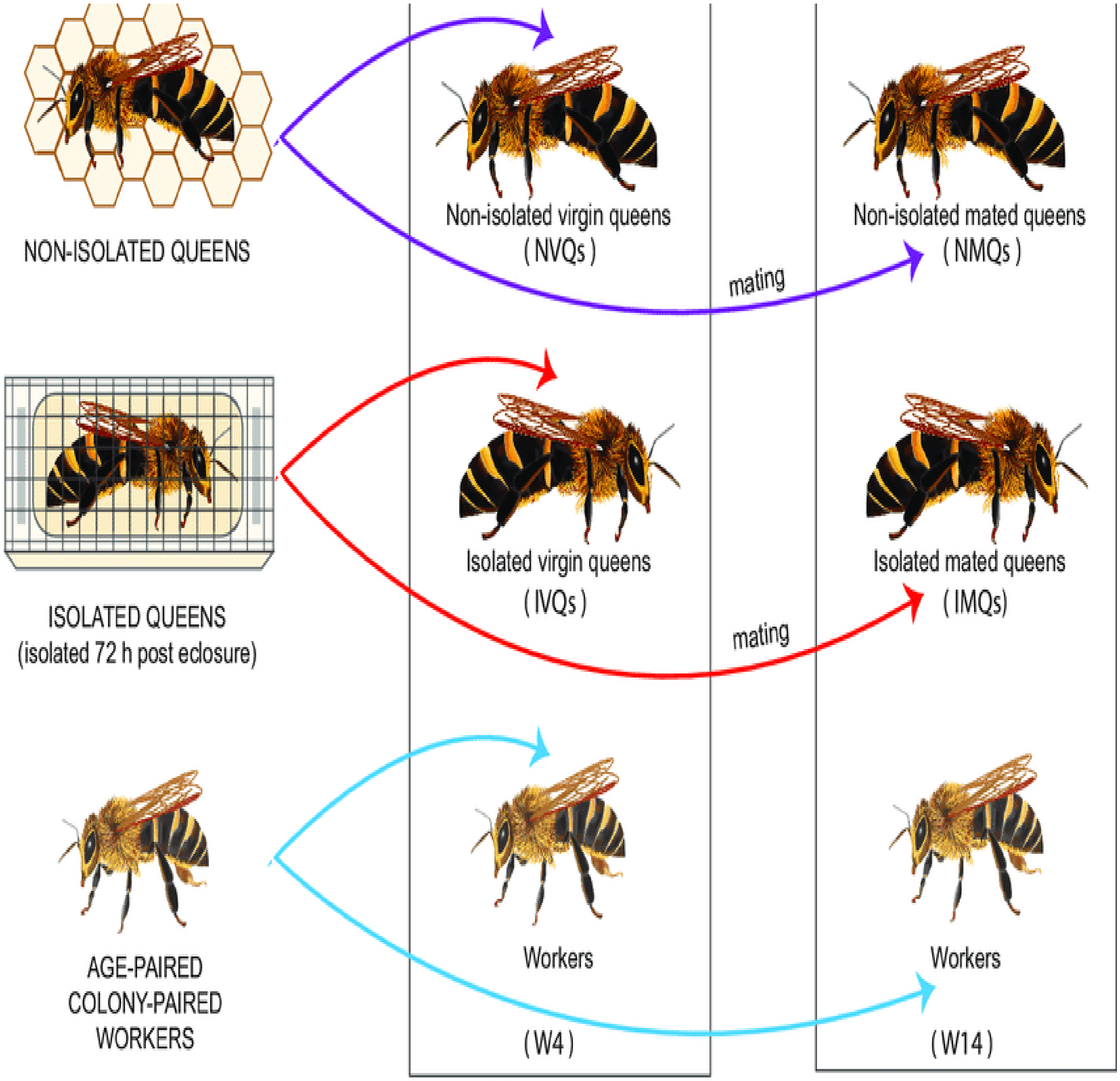
Furthermore the DWV an important impact on the brain regions in charge of fragrance had an adverse effect on honey bee foraging behavior .when compared with non-infected bees DWV infected bees fly a shorter distance and have shorter period of flight. When compared with non-infected honeybee's DWV-infected bees fly shorter distance and have less short distances and have less time to fly. The DWV affects the gene associated with expression that governs the memory wings creation and sensory perception. Additionally, CBPV grow aggregate, harms or disturb the sensory-processing neurons in the honeybee brain area (CNS).as a result the sick honey bee can only fly while creeping and buzzing at the colony opening (Traynor *et al.* 2020)

5: EFFECTS OF VIRUSES ON QUEEN BEE HEALTH:

The health of honey bees is very vulnerable to pathogenic viruses, which can also result in colony losses. In addition to disease-causing chemicals, the loss of the queen has been identified as one of the key causes of colony failure. Because the queen's presence is essential for a colony to coordinate duties and behavior through the release of pheromones and to lay eggs. Like to other colony members, the queen bee also picks up and experiences pathogenic virus attacks, and she can harbor many viruses (up to six at once). It has been established that infected drones can spread the DWV to queens by bringing contaminated semen to the mating location. Also, the discovery of viral titers in the drone, queen, and semen reproductive systems shows that sexual contact between bees can transmit viruses.



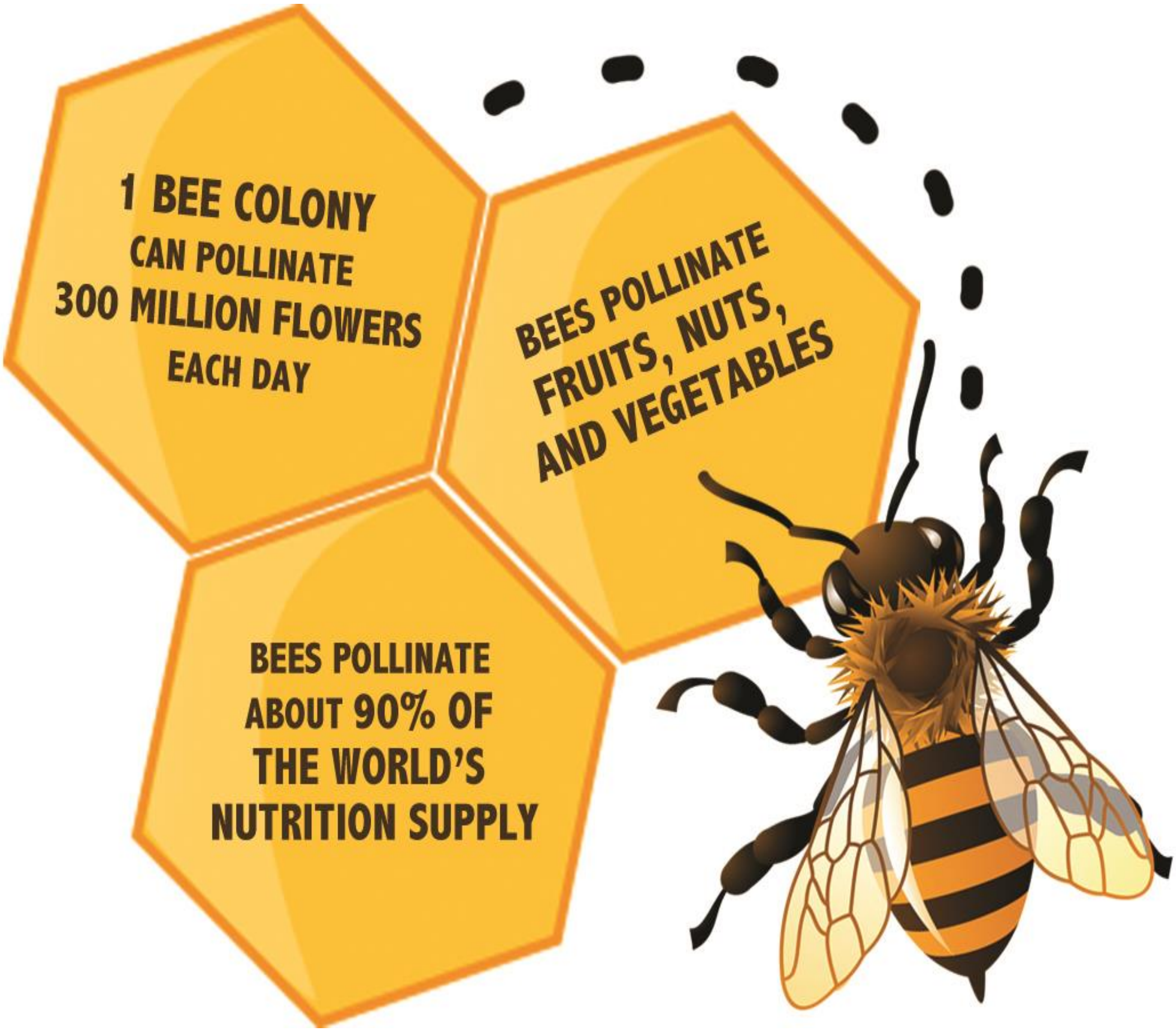
The brood of an infected queen can directly (vertically) receive virus particles. Vertical virus transmission happens when an egg is fertilized by infected sperms or through the already infected ovarian tissue of a queen bee. The digestive tract of the queen bee was found to include BQCV and DWV, according to a study led by Chen and his team. Although it is less common in young ones, DWV can infect both young and adult queens. It affects the intestines, head, ovaries, fat body, and can result in clinically obvious illness symptoms including distorted wings. Ovarian cancer is consequently brought on by the excessive viral infection in queen ovaries. Causes ovarian ageing and can be the reason for the death of implanted sperm (Sherman *et al.* 1998)



Hence, a decrease in reproductive capabilities could affect hive productivity and the need to replace the queen. SBV was also found in the ovaries and emboweled body of queen bees, although its transmission and harmful effects were not fully understood. BQCV has the potential to infect queen pupae and cause the death of honey bee queen larvae. BQCV particles were found in the queen bee's ovary and stomach. Additionally, BQCV, like other viruses, was found in A. melliferum all over the world, where it secretly infected adult and brood worker bees. The presence of DWV and BQCV in the digestive tract of the queen bee demonstrated that the viral titer must be ingested by the queen through contaminated food, providing a favorable environment for viral proliferation and allowing for easy transmission to other tissues (sperm theca, ovaries, and hem lymph). IAPV was found to be passed from infected workers to the queen bee by prophylaxis and direct contact with infected bees, according to recent findings (Lecocq *et al.* 2016)

6: VIRAL IMMUNITY'S IMPACT ON HONEY BEES:

The spread of viral particles from parasitic mites to honey bees reduces bee immunity or boosts the virulence and infectiousness of the virus. Moreover, the immunosuppressive proteins in V. destructor saliva intimidate infected honey bees' immune systems. The honey bee's mechanical defences are destroyed by the parasitic mite infection, leaving it vulnerable to additional viral assault. The strong virus infection promotes honey bee pathogenicity, suppresses their immunity, and ultimately renders them vulnerable to additional environmental pressures.



The olfactory learning of honey bees is also destroyed, and virus growth is facilitated by organ silicon spray additive (used in numerous pesticide formulations). Neonicotinoids also inhibit the functions of the immune and reproductive systems of bees, which increases the reproduction of DWV in bees. High DWV titers in bees have also been reported to suppress their immune responses and melanin production, which causes the spread of V. destructor mites and also cures its progeny. In addition, V. destructor septicemia sustains bee host immune suppression and increases the number of DWV infections(Williams *et al.* 2019)

CONCLUSION:

We have included a summary of the molecular diagnostics and genotyping methods that are now available for the identification and examination of the primary pathogens that affect the Western honeybee in this article. The selection of suitable tools for the proposed project can be aided by this overview investigation and information on a probable shortage of crucial chemical instrument be created. The molecular tools available are essential for I in-depth analyses of still-understudied or emerging bee pathogens, (ii) the identification of yet-unknown or cryptic species and clarification of their strain composition, (iii) research on co-infection with specific bee pathogens that synergistically increase pathogenicity, (iv) global surveillance of the most virulent pathogens, (v) the tracing of outbreak sources, and (v) a (e.g., A. tumida). The information is also intended to provide a comprehensive understanding of the unique characteristics of each disease and pathogenic group, as well as the interactions and similarities between them. The molecular diagnostic methods employed to research these infections and pathogenic groupings may, however, be compared and contrasted.

ACKNOWLEDGEMENT:

I would like to express my sincere gratitude to my dedicated managing director **Mr. Qamar-ul-Ikram** and Principal **Mr. Irfan Shafqat Javed** for his valuable suggestions, keen interest, guidance and inspiring attitude during my research project, and writing of this manuscript. I gratefully acknowledge invaluable help rendered by **Mr. Qasir Abdullah, Mr. Zahid Ali, Mr. Abdul Waheed** and **Asma Parveen** for guiding me at every step of the research work. They gave me time and helped me at every step in an effective development and conductance of research. I am honored to express my deepest sense of gratitude and profound indebtedness to all **"Aspire faculty"** for helping me to fulfill my research work. They combine a range of skills and experience, vision and commitment that are unequalled anywhere in the world.

REFERENCES:

Alaux C, Crauser D, Pioz M, Saulnier C and Le Conte Y. 2014. Parasitic and immune modulation of flight activity in honey bees tracked with optical counters. Journal of Experimental Biology. 217(19): 3416-3424.

Bird G, Wilson AE, Williams GR and Hardy NB. 2021. Parasites and pesticides act antagonistically on honey bee health. Journal of Applied Ecology. 58(5): 997-1005.

Gómez-Moracho T, Buendía-Abad M, Benito M, García-Palencia P, Barrios L, Bartolomé C, Maside X, Meana A, Jiménez-Antón MD and Olías-Molero AI. 2020. Experimental evidence of harmful effects of Crithidia mellificae and Lotmaria passim on honey bees. International Journal for Parasitology. 50(13): 1117-1124.

Grozinger CM and Robinson GE. 2015. The power and promise of applying genomics to honey bee health. Current opinion in insect science. 10: 124-132.

Kojima Y, Toki T, Morimoto T, Yoshiyama M, Kimura K and Kadowaki T. 2011. Infestation of Japanese native honey bees by tracheal mite and virus from non-native European honey bees in Japan. Microbial ecology. 62: 895-906.

Lecocq A, Jensen AB, Kryger P and Nieh JC. 2016. Parasite infection accelerates age polyethism in young honey bees. Scientific reports. 6(1): 1-11.

Liu Q, Lei J, Darby AC and Kadowaki T. 2020. Trypanosomatid parasite dynamically changes the transcriptome during infection and modifies honey bee physiology. Communications biology. 3(1): 51.

Liu Q, Lei J and Kadowaki T. 2019. Gene disruption of honey bee trypanosomatid parasite, lotmaria passim, by CRISPR/Cas9 system. Frontiers in Cellular and Infection Microbiology. 9: 126.

Matthijs S, De Waele V, Vandenberge V, Verhoeven B, Evers J, Brunain M, Saegerman C, De Winter PJ, Roels S and de Graaf DC. 2020. Nationwide screening for bee viruses and parasites in belgian honey bees. Viruses. 12(8): 890.

Meixner MD, Francis RM, Gajda A, Kryger P, Andonov S, Uzunov A, Topolska G, Costa C, Amiri E and Berg S. 2014. Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. Journal of Apicultural Research. 53(2): 215-229.

Nagaraja N and Rajagopal D**.** 2019. Honey Bees: diseases, parasites, pests, predators and their management. MJP publisher. p.

Ngor L, Palmer-Young EC, Nevarez RB, Russell KA, Leger L, Giacomini SJ, Pinilla-Gallego MS, Irwin RE and McFrederick QS. 2020. Cross-infectivity of honey and bumble bee-associated parasites across three bee families. Parasitology. 147(12): 1290-1304.

Paris JL, Villaverde G, Gómez-Graña S and Vallet-Regí M. 2020. Nanoparticles for multimodal antivascular therapeutics: Dual drug release, photothermal and photodynamic therapy. Acta biomaterialia. 101: 459-468.

Paris L, El Alaoui H, Delbac F and Diogon M. 2018. Effects of the gut parasite Nosema ceranae on honey bee physiology and behavior. Current opinion in insect science. 26: 149-154.

Ramasamy M, Lee J-H and Lee J. 2017. Direct one-pot synthesis of cinnamaldehyde immobilized on gold nanoparticles and their antibiofilm properties. Colloids and Surfaces B: Biointerfaces. 160: 639-648.

Ribani A, Utzeri VJ, Taurisano V and Fontanesi L. 2020. Honey as a source of environmental DNA for the detection and monitoring of honey bee pathogens and parasites. Veterinary Sciences. 7(3): 113.

Shende P and Narvenker R. 2021. Herbal nanotherapy: A new paradigm over conventional obesity treatment. Journal of Drug Delivery Science and Technology. 61: 102291.

Sherman PW, Seeley TD and Reeve HK. 1998. Parasites, pathogens, and polyandry in honey bees. The American Naturalist. 151(4): 392-396.

Siddique S and Chow JC. 2020. Gold nanoparticles for drug delivery and cancer therapy. Applied Sciences. 10(11): 3824.

Simone-Finstrom M. 2017. Social immunity and the superorganism: Behavioral defenses protecting honey bee colonies from pathogens and parasites. Bee World. 94(1): 21-29.

Simone-Finstrom MD and Spivak M. 2012. Increased resin collection after parasite challenge: a case of self-medication in honey bees? PloS one. 7(3): e34601.

Sulaiman GM, Waheeb HM, Jabir MS, Khazaal SH, Dewir YH and Naidoo Y. 2020. Hesperidin loaded on gold nanoparticles as a drug delivery system for a successful biocompatible, anti-cancer, anti-inflammatory and phagocytosis inducer model. Scientific reports. 10(1): 9362.

Tejeda-Serrano M, Cabrero-Antonino JR, Mainar-Ruiz V, López-Haro M, Hernández-Garrido JC, Calvino JJ, Leyva-Pérez A and Corma A. 2017. Synthesis of supported planar iron oxide nanoparticles and their chemo-and stereoselectivity for hydrogenation of alkynes. ACS Catalysis. 7(5): 3721-3729.

Tong Y, Xu S, Huang L and Chen C. 2022. Obesity and insulin resistance: Pathophysiology and treatment. Drug Discovery Today. 27(3): 822-830.

Traynor KS, Mondet F, de Miranda JR, Techer M, Kowallik V, Oddie MA, Chantawannakul P and McAfee A. 2020. Varroa destructor: A complex parasite, crippling honey bees worldwide. Trends in parasitology. 36(7): 592-606.

Tripathy B, Sahoo N and Sahoo SK. 2021. Trends in diabetes care with special emphasis to medicinal plants: Advancement and treatment. Biocatalysis and agricultural biotechnology. 33: 102014.

Unuofin JO and Lebelo SL. 2020. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. Oxidative medicine and cellular longevity. 2020.

Vyas V and Lambiase P. 2019. Obesity and atrial fibrillation: epidemiology, pathophysiology and novel therapeutic opportunities. Arrhythmia & electrophysiology review. 8(1): 28.

Williams M-KF, Tripodi AD and Szalanski AL. 2019. Molecular survey for the honey bee (Apis mellifera L.) trypanosome parasites Crithidia mellificae and Lotmaria passim. Journal of Apicultural Research. 58(4): 553-558.

Zhang D, Ma X-l, Gu Y, Huang H and Zhang G-w. 2020. Green synthesis of metallic nanoparticles and their potential applications to treat cancer. Frontiers in Chemistry. 8: 799.

Zhao G, Ran X, Zhou X, Tan X, Lei H, Xie X, Yang L and Du G. 2018. Green synthesis of hydroxylatopillar [5] arene-modified gold nanoparticles and their self-assembly, sensing, and catalysis applications. ACS Sustainable Chemistry & Engineering. 6(3): 3938-3947.