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Biodiversity and Challenges of Honey Bee Population in Pakistan

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Abstract

Honey bees are important pollinators that support food security and nature's biodiversity. They are also a source of various honey bee-derived products (api-products) used in the food, pharmaceutical, and cosmetic industries. However, various biological, chemical and physical factors threaten the population and biodiversity of feral and managed honey bees. These challenges have not been elaborated upon in the Pakistani context; therefore, this review aims to identify and describe the menaces to feral and domesticated populations of honey bees in Pakistan. Four honey bee species are reported in the country, with the Western honey bee (*Apis mellifera*) currently being the main domesticated species. Climate change and urbanization are altering the habitats of honey bees. Additionally, agrochemicals are extensively used to manage emerging pests, exacerbating environmental pollution. The air quality in the majority of urban areas is toxic for honey bees. Although remote forest areas can provide habitat and food for these insects, low forest cover and non-sustainable silviculture are still significant hurdles. Microplastics and antimicrobials are impacting the fitness of honey bees and also appear in their products, making it a One-Health issue. Electromagnetic signals also influence honey bee health and behavior. Overall, all these factors influence honey bee health and colony fitness, ultimately causing population declines in both managed and wild honey bees. The purpose of this information is to assist decision-makers, researchers, beekeepers and educators in comprehending the obstacles faced by the honey bee population within the context of Pakistan.

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Introduction

Honey bees, as invaluable social insects, play a crucial role in providing essential pollination services worldwide, thereby enhancing agricultural productivity, ensuring food security, and fostering vegetative biodiversity. Moreover, honey and various products derived from bees, including beeswax and propolis, hold considerable economic importance and find applications in the food, cosmetics, and medical sectors [1, 2]. Historically, beekeepers in the mountainous regions of Pakistan maintained colonies of *Apis cerana* for honey production. Nevertheless, between 1980 and 1983, a substantial portion of these colonies perished due to an epidemic, likely caused by tracheal mites [3]. In this era, various programs were launched to use Western honey bees (*A. mellifera*) for apiculture. In 2008, approximately 2,000 beekeepers were culturing nearly 200,000 to 300,000 *A. mellifera* colonies, resulting in an annual honey yield of 7,500 tons [3]. Presently, this number has escalated to 10,000 beekeepers keeping approximately 0.5-1.1 million colonies and yielding 16,000 tons of honey [4]. The domestic honey consumption in Pakistan is more than 11,000 tons, *i.e*., 50 g/capita [5]. The export, mainly to Arab countries, is 4,000 tons, bringing about US$ 23 million to the local Gross Domestic Product [6]. Pakistan is the 5th most populous country in the world and ranks 20th in the world for honey production and 34th in honey export. Meanwhile, an average Pakistani beekeeper generates around 11.7 kg of honey, contrasting with the global average of 20.6 kg [7]. Therefore, the ever-increasing demand for honey bee products in the world provides an opportunity for developing countries such as Pakistan to utilize the product production potential to support their economies.

The health and biodiversity of honey bees are threatened by diverse biotic and abiotic factors [1, 8–11]. In the last six decades, the global number of managed honey bee colonies has increased by 85%, honey production by 181% and beeswax production by 116%. However, this increase is still lower compared to human population growth during this period which is about 50% in the world [12] and more than 500% in Pakistan. Meanwhile, the number of honey bee colonies per capita is continuously decreasing around the globe [12], for example, Europe and United States have observed a 30% reduction in managed honey bee colonies in the last decade [13]. Moreover, the main effect has been the loss of wild honey bee populations and their diversity which are equally affected by the challenges of pesticides, environmental pollutants, emerging biological hazards, climate change, deforestation, etc. [13, 14]. This declining population is a menace to agricultural productivity and can potentially result in a food security crisis. This literature review provides information on honey bees and apiculture growth in Pakistan. It includes both the feral and managed honeybees. Further, it discusses the role of different biotic and abiotic factors on the honey bee population in Pakistan. Due to data limitations, we have enlisted different factors affecting the honey bee population globally and then discussed them in the Pakistani scenario. To our knowledge, there is no comprehensive literature review published in this specific context. This knowledge will provide guidelines to researchers, beekeepers, and biologists to better understand the challenges and protect the honey bee populations in Pakistan.

Significance of honey bees

Preserving pollination and biodiversity

Honey bees play a vital role in nature, particularly in pollination and sustaining vegetative biodiversity, which are crucial components of the Earth's living fabric. About 80% of pollination on our planet is performed by bees [15]. There are 61 main honey bee pollination-dependent food crops in Pakistan, including 26 fruits, 19 vegetables, seven oilseeds, four leguminous grains, two flavoring crops, and three nut shrubs [1]. However, owing to increased cropping practices and decreased honey bee populations, the current honey bee population is regarded as far too insufficient to supply optimum pollination services in global agricultural systems [16].

Honey bee products

Many nutritional and medicinal products are obtained from honey bees (Table 1). The demand for these products is increasing, especially minimally processed natural products.

Environmental monitoring

Pollutants contaminate the living and non-living entities of the environment. The same is true for honey bees when they are exposed to various chemicals during foraging or feeding. The contaminants become part of their body and likewise, they are excreted in their products also [23]. For instance, honey bees collect pesticide residues, heavy

Table 1 Honey bee-derived products and their uses.

Products	Description	Properties and uses	References
Honey	A sweet, acidic (pH 3.5-4), and viscous substance (more than 180 ingredients) produced by honey bees from the nectar of flowers.	Nutritional, medicinal (healing, anti-inflammatory and antimicrobial)	[2, 17]
Venom	Produced in the poison glands in the abdominal cavity of worker honey bees, venom is a mixture of enzymes, proteins, and peptides containing more than 60 identifiable compounds	Antibacterial, fungicide, antiviral, anti-inflammatory, antiarthritis, antitumoral, and anti-neurodegenerative	[18]
Propolis/ bee glue	It is a resinous blend created by bees through the combination of saliva and beeswax with exudate collected from tree buds, sap flows, or other botanical origins. It contains over 300 active compounds.	Antiseptic, anticancer, antiulcer, anti-inflammatory, antibacterial, antimycotic, antifungal, antioxidant, and immunomodulatory bioactivities	[19]
Hive/ Beeswax	The ventral glands on the worker bees' abdomen produce wax that is composed of hydrocarbons, esters, free acids, and other compounds.	Candles and water-repellent cosmetics and polishes. It also has antibacterial anti-inflammatory activities	[20]
Pollen	Bee pollen is a mixture of pollen, nectar, enzymes, honey and wax that bees collect from flowers. It has more than 200 biologically active substances.	Dietary supplements claimed to have immune-booster, anti-inflammatory, and antiallergic benefits	[21]
Royal jelly	It is a thick, milky, nutritious secretion that is produced by worker honey bees to feed the queen bee and developing larvae	Anti-inflammatory, immunostimulatory, antimicrobial, antitumor, hypotensive, antioxidant, and wound-healing substances	[22]

metals such as arsenic, cadmium, and lead, among other chemicals from their surroundings and transfer them to products [24]. As a result, honey bees and their products can serve as bioindicators for environmental monitoring. The quantitative analysis of contaminants such as heavy metals, radionuclides, pesticides, explosives, nanoparticles, organic pollutants, and phytopathogens in honey bees and their products sensitively reflects environmental and plant health [25]. Numerous studies have documented the presence of antimicrobial resistance genes in honey bees and their products, indicating the excessive use of antibiotics in apiculture and environmental contamination by antimicrobial-resistant microorganisms [26, 27].

Factors affecting honey bee population and diversity in Pakistan

Climate change, deforestation and habitat loss

The rapid pace of climate change is transforming landscapes and environments in unprecedented ways. Pakistan stands out as one of the countries, most profoundly affected by these shifts. These intricate circumstances are reshaping the habitats where both wild and managed honey bees seek food and refuge. For instance, unusually heavy rains and cooler temperatures in Pakistan's northern regions are causing delays in flowering. This situation also reduces the foraging activities of bees, ultimately lowering honey production [14]. In October 2014, Pakistani beekeepers were able to harvest only 20% of the berry honey. The reason behind this was the sudden rain and wind storms during the middle of the honey flow period. This changed weather pattern not only caused the drop of nectar-containing flowers but also took away the colonies [14]. Meanwhile, sudden flood situations are also causing honey bee colony losses in Pakistan [28]. Climatic changes impact the foraging behavior of honey bees [29]. Farooqi et al. [9] studied the impact of abiotic factors (light, temperature, wind, humidity) on the foraging behavior of two native honey bee species (*A. dorsata* and *A. florea)* in Bahawalpur, Punjab-Pakistan. They observed that nectar-robbing events were unaffected under any tested situation for *A. dorsata*. However, all the changing factors negatively influenced the visitation frequency of these honey bees. Stigma contact time also decreased at higher temperatures and increased with light intensity. On the other hand, In the case of *A. florea*, stigma contact was not influenced by tested abiotic factors. Visitation rate and stay time changed with light intensity. Nectar-robbing increased at higher relative humidity and decreased at stronger wind speeds. Jaworski et al. [29] reported that drought conditions negatively influenced the foraging behavior of honey bees. Therefore, the deviations in the environmental factors under seasonal pattern changes significantly influence the behaviors of honey bees.

Changing climatic and seasonal patterns can influence the ability of honey bees to adapt to seasons and sustain their normal physiology. For example, hypopharyngeal glands in worker and forager honey bees undergo physiological and morphological changes in the winter and summer seasons, and it changes their ability to produce honey [30]. Long-term changes in the endocrine system under environmental stress ultimately challenge the colony fitness and survival of honey bees [31]. The honey populations decline in the absence of flowering season. This is one of the reasons behind winter and extreme summer die-off in honeybees. In these situations, the domesticated honeybees should be fed supplementary diets to fulfill their nutritional requirement for young bee's development, brood rearing, reproduction, and maintenance of bee colony and honey production [32]. Rapid urbanization and demand for land resources have destroyed the wild environments on which bees depend, for example, we lost wilderness areas, meadows, and hedgerows and it has considerably impacted all wildlife, including bees. Urbanization changes biotic community composition by creating a matrix of habitats distinct from natural ecosystems [33]. Honey bees are important for biodiversity, and vegetative biodiversity is important for honey bees. Modern commercial agriculture involves the plantation of mono-crops leading to the depletion of plant diversity. Honey bees do not forage the same on different crops, for example, honey bees can collect only pollens from palm oil and corn crops and only nectar from the rubber trees. St. Clair et al. [34] reported that honey bees kept at diversified farms had increased colony weight and pre-overwintering nutritional state compared to when they were foraging on monoculture crops. de Groot et al. [35] reported that the industrial mono-crop culture of soybean has decreased honey yield in Argentina by 60%. Moreover, the honey bees cannot keep pace with the winter die-off rates and habitat loss due to monoculture. Therefore, incorporating natural and diverse crops is of great importance for apiculture sustainability and honey bee conservation efforts [15].

Apiculture practices

There are four honey bee species reported in Pakistan: the rock honey bee (*A. dorsata* F.) locally known as Dumna or Bari Makhi, dwarf honey bee (*A. florea* F) known as Choti Makhi, Asian hive honey bee (*A. cerana* F.) locally known as Pahari Makhi and Western honey bee (*A. mellifera* L.) [36] (Fig. 1). However, the increase in the number of colonies of honey bees in Pakistan is mainly limited to Western honey bees. There are some subspecies of *A. mellifera* also reported in Pakistan based on their morphologic and genetic analysis [37–39]. In general, there is a decline observed in all managed and wild honey bee populations. Concurrently, the culture of a singular honey bee population for beekeeping poses a significant threat to genetic diversity in both wild and managed populations, potentially exacerbating the decline in population numbers [40]. Studies have shown that a solitary honey bee population could establish dominance within an ecosystem, leading to a disproportionate exploitation of flowering plant resources. According to Hung et al. [41], honey bees exhibit a preference for high-abundance floral resources due to their capacity to recruit nest-mates. Consequently, this dynamic may compel other indigenous pollinator insects to forage on less abundant resources in order to mitigate competition. Likewise, the introduction of exotic bees to different new areas creates an overlapping competition for native bee populations to utilize the resources of the area [42, 43]. Here it is important to mention the competition for resources is not only limited to inter-species competitions but also the density of one species in a given region. Henry and Rodet [44] reported that high-density apiculture induces foraging competition which depresses not only the occurrence (−55%) and nectar foraging success (−50%) of local wild bees but also nectar (−44%) and pollen (−36%) harvesting by the honey bees themselves. As there are at least four honey bee species in Pakistan, research

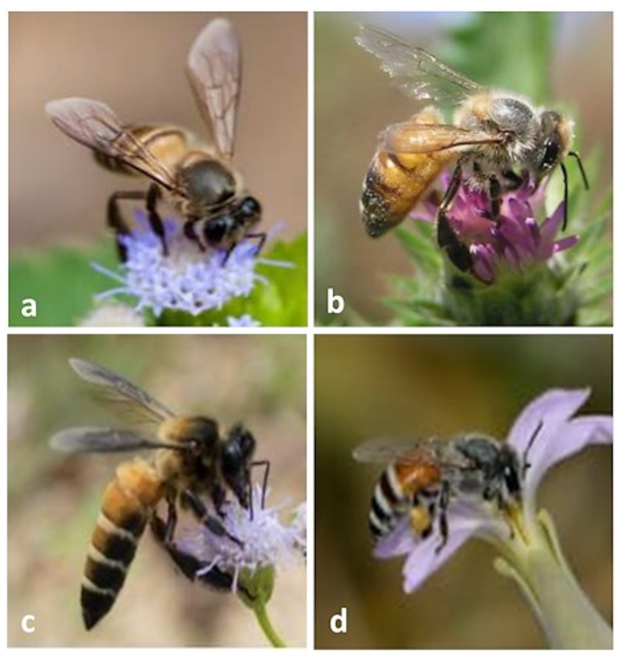

Fig. 1 Honey bee species in Pakistan. (a) *Apis cerana*, (b) *Apis mellifera*, (c) *Apis dorsata*, (d) *Apis florea.*

is needed to elucidate the interspecies and inter-genus interactions of these bees in the same ecosystem. Bee hunting plays a significant role in honey production in Pakistan. However, uncontrolled hunting methods, such as hive destruction and complete nest removal (including honey and brood), could exert pressure on wild bee populations.

Chemical hazards

Honey bees are exposed to various agrochemicals and pollutants of the environment during their routine activities (Fig. 2). These chemicals cause direct contamination of pollens and guttation drops through aerosol [45]. Soil, plant and water source contamination leads to the absorption of these chemicals by plants and becoming part of the nectar [46]. Ultimately, the contaminated pollens and nectars foraged by honey bees put them at risk of absorbing harmful chemicals. Honey bees are also directly exposed to these hazards while flying through contaminated air or drinking contaminated water [47, 48]. Once introduced into insects, these stressors can induce, alter, or inhibit a range of biological responses and metabolic pathways, depending upon the type and dose of the chemicals. In general, chemical toxicity changes the cognition and behavior of honey bees [46]. Morphological changes have also been reported [49, 50]. They are also reported to be one of the causes of colony collapse disorder in honey bees [48]. Di Noi et al. [51] reported the sublethal effects of different environmental pollutants as contaminant accumulation, foraging activity, enzymatic and molecular responses, detoxification, neurotoxicity, metabolic responses, immunity, and oxidative stress. The combination of biological and chemical hazards also results in an important disorder of honey bees called colony collapse disorder (CCD) [1].

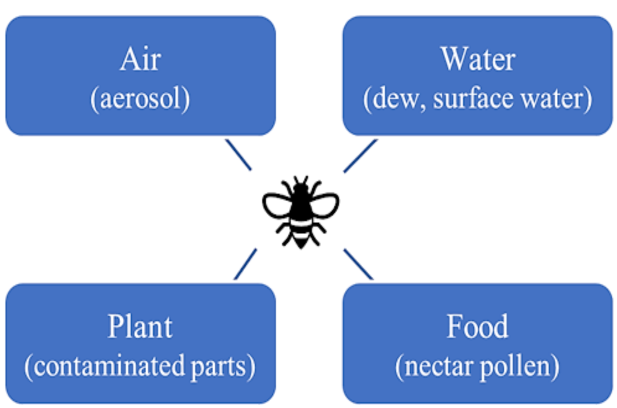

Fig. 2 Exposure sources of bees to different chemical hazards.

Particulate matter (PM) and air pollutants

Air particulate matter has different compositions based on the type and concentration of chemical contaminants. It is classified by size ranging from a few nanometers ($PM_{0.1}$) to several micrometers (PM_{10}). During the flight and foraging activities, honey bees are directly exposed to these chemicals as respirable suspended PM [52]. Meanwhile, they can also bring the PM to their hives by contact because during their flight the pubescence promotes the accumulation of electrical charge on the body surface, which increases airborne PM attraction [53]. The foraging behavior of honey bees changes during high air pollution [52]. There are two main reasons for this change, firstly, the higher particulate matter decreases sunlight intensity which is used by honey bees for navigation [54]. Secondly, the ecologically relevant concentrations of common urban air pollutants influence the olfactory learning and memory in honey bees which are known to play significant roles in foraging [55, 56].

Urban areas in Pakistan experience significantly high levels of air pollution, with even Islamabad, considered one of the least polluted cities, exceeding national air quality standards for annual average mass concentration of particulate matter ($PM_{2.5}$) (~45 to ~95 $\mu g\ m^{-3}$) and nitric oxide (NO) (~41 to ~120 $\mu g\ m^{-3}$) [57]. Khan et al. [58] investigated the impact of polluted PM on the behavior, foraging patterns, and brood development of Western honey bee (*A. mellifera* L.) within Pakistan's agro-industrial ecosystem, finding that PM contamination adversely affected honey bee health and behavior, leading to reduced hive populations, smaller brood sizes, and diminished honey storage [58]. In many parts of the country, the zone (O_3) contamination reaches up to 370 ppb [59], which is dangerous for honey bees. It has been reported that the O_3 at 80-120 ppb decreases the recall of learned odor association antennal activity in honey bees. Another air pollutant, SO_2 has been reported to be within permissible limits (15.60–110.52 $\mu g/m^3$) in the major cities of Pakistan [60], however, these levels significantly affect the foraging behavior of honey bees [61,62]. The PM contains many other pollutants which could harm honey bee health. Papa et al. [48] reported that the acute and chronic oral administration of ultrapure TiO_2 PM_1 to adult bees altered the bee microbial community and thus could be a risk factor for honey bee's health.

Potentially toxic elements

Metal pollutants originating from industrial waste, sewage, fertilizers, and pesticides infiltrate soil, air, and water bodies [63], with no part of the earth being entirely devoid of anthropogenic contaminants [25]. Honey bees are exposed to these pollutants via contaminated air, water, nectar, and pollen. Similar to other chemical exposures, a multitude of intrinsic and extrinsic factors influence the exposure and impact of these pollutants on honey bees. The abundance of pollutants positively increases the risk of exposure, for example, heavy metal pollution of the environment has increased with the urbanization of geographical areas and the same trend has been observed in the accumulation of Pb in honey bees [64]. However, the uptake of different toxic elements by honey bees can vary even within the same hive [11]. Meanwhile, they do not sense the presence of ecotoxicological risks in their food. Monchanin et al. [65] reported that in a laboratory assay, bees did not discriminate food contaminated with arsenic, lead, or zinc and ingested it readily, up to estimated doses of 929.1 μg g^{-1} As, 6.45 mg g^{-1} Pb and 72.46 mg g^{-1} Zn. These environmental pollutants impact honey bee health in various ways. Heavy metals such as arsenic, lead, and copper can impact the cellular functioning of honey bees either by mimicking other essential metals or directly affecting signaling pathways and homeostasis [47,66]. In addition, these metals have additive effects creating a high cumulative hazard [47].

The soil, surface, and ground water are contaminated with different element pollutants in Pakistan [67,68]. A study from Pakistan reported that the majority of rivers in the country are contaminated with heavy metals e.g., Mn, Co, Cu, Zn, Cr, Ni, Cd, Hg, and Pb [69]. Some contaminations exceed the limits recommended for human consumption. Hayder et al. [70] reported that about half of the water resources in the northern region of Pakistan have high contamination of Pb and Cr (0.04 mg/L and 0.06 mg/L) which is higher than the safe drinking water guidelines of the WHO. Shakir et al. [71] also reported the high contamination of rivers, soil, and air by Hg, As, Pb, Cd, Zn and Fe oxides, Ni, Cr, Cu, Co, and Mg. These studies have reported that the cumulative hazard of these elements to human health is high. Similar can be applied to the honey bees, for example, Monchanin et al. [72] reported that the heavy metal pollutants of the environment can have additive effects on the learning and cognitive functions of honey bees. Moreover, it has been reported that heavy metal pollutants (As, Cd, Pb, and Hg) impact the honey bees even at levels below those recommended as ‘safe’ for humans [47]. Overall, many studies have shown the contamination of api-products in Pakistan [19, 73–75], indicating that honey bees are regularly exposed to these hazardous elements.

Pesticides

Pesticides are used to control plant pests. However, many of these chemicals can also harm the beneficial pollinators, including honey bees. These compounds have sublethal, and lethal effects on honey bees depending upon the type of chemical, exposure dose, exposure route, simultaneous toxicity by other pollutants, honey bee lifecycle stage, type of bees, and the environmental conditions [45, 76, 77]. Anwar et al. [78] investigated the 48-hour exposure toxicity of various insecticides on the Pakistani dwarf honey bee. They observed that emamectin benzoate exhibited the highest toxicity with an LD_{50} value of 1.02 μg/mL, followed by spinetoram (1.10 μg/mL), chlorantraniliprole (2.74 μg/mL), imidacloprid (3.09 μg/mL), flonicamid (3.94 μg/mL), and fipronil (6.00 μg/mL). Another study conducted in Pakistan revealed that the LD_{50} of neonicotinoid (imidacloprid) was the lowest (0.477 ng/bee) compared to carbamate (carbaryl) and organophosphate (chlorpyrifos) in *A. mellifera* L. honey bees via feeding bioassay [79]. *A. cerana* generally exhibits greater sensitivity to insecticides (imidacloprid and clothianidin) compared to A. mellifera [80]. Likewise, Liu et al. [81] also reported that the acute toxicity values (LD_{50}) of four pyrethroid pesticides to the *A. mellifera* L. were higher than of that the *A. cerana* C. Moreover, the survival rate of *A. mellifera* L. (40.0%) was also higher than the *A. cerana* C. (18.9%) when exposed to the same concentration of beta-cypermethrin (0.2906 mg/L). Pesticide combinations can have additive and synergistic lethal effects on honey bees [82,83]. Almasri et al. [82] reported that the combination of insecticide (imidacloprid), fungicide (difenoconazole), and herbicide (glyphosate) had synergistic negative effects on the health of winter honey bees. Environmental conditions such as the weather can also influence the susceptibility of honey bees to different pesticides. Saleem et al. [77] observed that Western honey bees were significantly more sensitive to the neonicotinoid pesticides (imidacloprid and thiamethoxam) when exposed at a constant (24 °C) or a varying temperature (night at 13 °C and day at 24 °C) compared to when exposed at high temperature (35 °C).

Pesticide use in Pakistan has increased 12-fold since the start of this century. The quantities of the major pesticides used in the country are in the following order: pyrethroids > organophosphates > organochlorines > carbamates [84]. Moreover, various neonicotinoid pesticides are also used in Pakistan [79], even though they were banned in the European Union owing to their harmful effects on honey bee health [85]. Meanwhile, an increase in resistance to pesticides in many pests is being reported in Pakistan [86]. Due to high pest resistance, new chemicals are being introduced in the Pakistani market thus exposing honey bees to more variety of chemical risks [87].

Apart from the systemic use of pesticides to control herbs, insects, and fungi in crops, insecticides have been extensively used in Pakistan to curb recent locust infections [88]. For example, during the 2018-2020 locust plague, about 5.5% area of Pakistan was treated with low doses of insecticides (fenitrothion, malathion, diazinon, deltamethrin, bendiocarb, cyhalothrin, diflubenzuron) [89]. Many other chemicals have been used in recent times as disinfectants and insecticides to control mosquito-borne diseases and COVID-19 transmission. For example, dodecyl dimethyl benzyl ammonium chloride (DDBAC) was used abundantly as a disinfectant against the contamination of SARS-CoV2. However, this chemical is toxic to honey bees and has been reported to disrupt gut microbiota, phospholipids, and calcium signaling in *A. mellifera* honey bees at an environmentally relevant level [90]. Overall, honey bee populations across Pakistan face extensive pesticide exposure. Farooqi et al. [91] investigated insecticide residues in the honey of *A. dorsata* F. from Southern Punjab, Pakistan. Their findings revealed widespread presence of imidacloprid, endosulfan, and deltamethrin residues in the samples, with concentrations ranging from 5 to 55 µg/kg. A study from northern Pakistan also showed the contamination of honey with many organochlorine insecticides, including the ones that have been banned in Pakistan [92]. Therefore, regular monitoring of the products can give information on the exposure of honey bees to different pesticides. It is also recommended to use integrated management practices for the control of pests in the agriculture field and should not only rely on chemical control measures and use fewer toxic chemicals in the low honey bee activity periods [1,93].

Fertilizers

Fertilizers can adversely affect bees by influencing the plants upon which bees depend for food. When fertilizers are applied to crops or plants, their metabolites and metal pollutants can contaminate the nectar and pollen of these plants, diminishing their nutritional content and potentially posing toxicity to nectar-feeding organisms [94,95]. Even though flower morphology and smell changed by fertilizers do not seem to affect the foraging behavior of honey bees, these chemicals can modify the complex set of interlinked biophysical properties of the flower thus affecting the behavior of bees [96]. All these factors cumulatively result in reduced bee health and reproductive success, and can also lead to population declines. In addition, fertilizers can contribute to the pollution of waterways and other natural habitats, which can negatively impact the ecosystems that support honey bee populations. Overall, the type and source of fertilizer differently affect honey bee health and behavior [97]. The fertilizer-associated nitrogen levels also cause environmental pollution by forming ammonia, NOx, and ozone gasses which have been reported to be toxic to human and honey bee health [98,99]. Rollin et al. [100] reported that at high air ozone levels, the strength of the effect of pesticides on honey bees was more than double compared to when the ozone levels were intermediate.

Pakistani agriculture extensively relies on fertilizers because of nitrogen and phosphorus deficiencies in agricultural soils. The most commonly used fertilizers in Pakistan are urea, di-ammonium phosphate (DAP), and potash which contain nitrogen, phosphorus, and potassium nutrients [101]. In the majority of cases, fertilizers are used without proper testing of soil requirements which results in an imbalance of nutrients in the soil and pollutes the air. For example, it has been reported that Pakistan is facing a continuous decline in nitrogen use efficiency which was 58% in the 1960s and only about 23% in the 2010s [102]. In such overuse cases, the nutrients leach into the soil and groundwater, causing pollution and environmental degradation [103]. Tahir and Rasheed [104] reported that about 20% of water samples collected from different parts of Pakistan were contaminated with harmful levels of fertilizer-origin nitrites (>10 mg/L). Meanwhile, it has also been reported that the positive effects of honey bee pollinators on crop yield are most accentuated under low inputs of nitrogen (e.g. ≤72 kg/ha) [105].

Microplastics (MPs)

The small particles of different plastics with a diameter of less than 5 mm are regarded as microplastics (MPs). They have already largely contaminated water, air, and soil around the globe [106] and the Pakistani environment is no different in being contaminated with MPs [107–110]. Feeding, on the contaminated water and nectar, and even flying through the contaminated air exposes honey bees to MPs. Ultimately, these MPs containing polyethylene, polypropylene, and polyacrylamide polymers, become part of api-products [8]. Studies have shown that about two-thirds of the bee samples exhibited the presence of four types of MPs [111]. To our knowledge, there have been no studies published in Pakistan regarding MP contamination in honey bees and products.

Studies have shown that exposure to various MPs can cause deleterious health effects in honey bees. Exposure to MPs has been reported to alter the honey bee gut microbiota (dysbiosis), followed by changes in gene expression related to oxidative damage, detoxification, and immunity [8,112]. Balzani et al. [113] reported that polyethylene MPs (0.5-50 mg/L) affected bee survival and feeding in a dose-dependent manner. However, they did not find any effect of MPs on the associative and non-associative learning or memory of honey bees. Alma et al. [24] reported that one-month exposure to MPs (50 mg/L) does not influence the hive populations. However, the MPs ingested and attached to honey bees are transferred to honey, wax, and larvae. Buteler et al. [114] reported that the acute exposure of polyester MPs through feeding in sucrose solution (100 mg/L) did not influence the survival of honey bees. However, honey bees were unable to differentiate the contaminated solution, which could make them prone to chronic effects of MPs in long-term consumption. Al Naggar et al. [115] reported that chronic exposure to polystyrene MPs at lower doses (10-100 µg/L) lowered the feeding rate and body weight of honey bees, however, the survival rate was not influenced. These effects can relate to not only the dose and type but also the size of plastic particles [111]. Wang and Zhou [116] reported that the orally administered polystyrene MPs (100 nm) adhered to the germination pore of pollen, while the larger particles (1- 10-µm) were attached by gut bacteria. Overall, the smaller particles significantly decreased the whole-body weight and survival rate of honey bees and induced intestinal dysplasia. They also reported that the relative abundance of *Lactobacillus* and *Bifidobacterium* in the guts was decreased, more immune inhibitory genes were expressed and genes related to detoxification and energy balance were depressed. Consequently, 100-nm MPs-treated honey bees became more susceptible to the pathogenic *Hafnia alvei*, leading to a five times higher mortality rate. Wang et al. [112] have reported the intestinal microbiome is damaged by MPs, and it became more severe when the honey bees were already treated with tetracycline. In another study, it was reported that ingestion of polystyrene MPs increased the susceptibility of honey bees to the Israeli acute paralysis virus (IAPV) which is associated with bee colony decline [111]. The researchers observed that the MPs accumulated in the midgut of honey bees and caused tissue damage, subsequently, these particles also transferred to the hemolymph, trachea, and Malpighian tubules. At the molecular level, genes correlated with membrane lipid metabolism, immune response, detoxification, and the respiratory system were significantly regulated after MPs ingestion [111].

Antibiotics

Antibiotics are employed in apiculture to address diverse infections in honey bees, yet their misuse can detrimentally impact bee health. These chemicals indiscriminately eliminate or inhibit the growth of various microorganisms, including beneficial microflora [117]. Anjum et al. [118] reported that the honey bees in the North-western region of Pakistan had many beneficial microbes in their gut and they are believed to act as symbiotes [118]. Lv et al. [117] reported that six *Limosilactobacillus reuteri* and one *Lactobacillus helveticus* isolated from gut samples of *A. cerana* antagonized the growth of pathogens including *Salmonella* Typhimurium, *Escherichia coli*, *Shigella flexneri*, and *Flavobacterium frigidimaris*. However, all these probiotic bacteria are sensitive to most antibiotics including ampicillin, amoxicillin, cefotaxime, cephalothin, kanamycin, novobiocin, penicillin G and vancomycin. Therefore, the use of antibiotics causes gut dysbiosis in honey bees, rendering them prone to many pathogenic organisms and toxicities [112]. Deng et al. [119] reported that long-term exposure to tetracycline, the most commonly used antibiotic in apiculture [120], changed the native gut microbiome of adult worker bees (*A. cerana* and *A. mellifera*). However, the extent of dysplasia was different depending on the honey bee species. More importantly, the antibiotic treatment significantly promoted *A. cerana* susceptibility to IAPV [119]. Wang et al. [112] reported that the gut microbiota depletion using tetracycline aggravated MPs toxicity in the *A. mellifera* L. This antibiotic has also been reported to cause a reduction in the digestion of pollens in honey bees [121] and elevated mortality [122]. The antimicrobials also cause gut microbiota changes and other health effects in the larvae and pupae [120,123].

For example, Duan et al. [124] observed that the antibiotics (penicillin–streptomycin) caused gut dysbiosis in honey bee larvae, resulting in lowered nutrient metabolism, decreased body weight, delayed developmental process, and weakened immunity. Aljedani, [120] reported that tetracycline (LC_{50} = 125.25 µg/ml) caused histological changes in the midget and decreased the bio-efficiency of the *A. mellifera* J. larvae. Another concerning consequence of antibiotic use is the development of antimicrobial resistance (AMR) in microbes, which makes honey bees vulnerable to infections that were previously treatable [125]. The AMR genes in honey bees either come from the environment or resistance is developed within the gut microbiota of honey bees when they are exposed to antibiotics in apiculture or uptake it from the contaminated environment [126,127]. The inheritance of antibiotic-acquired dysbiosis affects and AMR development can affect the phenotypes across generations [128,129]. The intestinal microbiome of honey bees may contain human and animal pathogens, e.g. *Salmonella enterica* and *Shigella sonnei* [130]. Development of AMR in these pathogens in the honey bee gut would pose a threat to human, animal, and honey bee health [131,132].

Biological hazards

The biological hazards to honey bees include bacteria, viruses, fungi, parasites, and other pests and predators. Various microorganisms and pests have been transferred from the wild to managed and managed to wild honey bees [133].

Bacterial diseases

Different bacteria cause infections in honey bees. The two main diseases are the American and European foulbrood which affect the *A. mellifera* larvae. American foulbrood is caused by *Paenibacillus larvae* (a spore-forming bacteria) and it is more dangerous compared to European foulbrood caused by *Melisococcus plutonius.* Both diseases have been reported in the northwestern part of Pakistan [134,135]. Some other opportunistic bacteria, such as *Serratia marcescens* can also cause infections in honey bees [136].

Fungal diseases

Several fungal diseases of honey bees have been reported in Pakistan [135,137]. The *Ascosphaera apis* fungi cause chalkbrood, characterized by the larvae turning grey or pale yellow and then dying turning black [135]. *Aspergillus flavus* and *A. fumigatus* cause stonebrood which is a rare and usually short-lived disease. This infection turns the larvae black and they are covered with powdery fungal spores and are difficult to crush [137]. *Vairimorpha apis* and *V. ceranae* (formerly classified under the Nosema genus) are spore-forming unicellular fungi responsible for inducing Nosemosis in adult Western honey bees. This condition leads to decreased productivity and reproduction. Approximately 25% of *A. mellifera* colonies in Pakistan have been reported to be contaminated with microsporidian spores [138].

Parasites

Varroa mite (*Varroa destructor*: Anderson and Trueman) is the most destructive ectoparasite in Pakistani apiculture [139]. It feeds on the fat bodies of adult bees, prepupae, and pupae and can transmit pathogens during parasitism. This is the natural pest of *A. cerana*, however, it also infested the Western honey bees when introduced in Pakistan in the 1980s [14]. The infestation of 0.64% of colonies has been reported in the Khyber Pakhtunkhwa province of Pakistan [140]. Tracheal mite (*Acarapis woodi*) is an internal parasite found in the trachea and air sacs of honey bees and feeds on the hemolymph or blood. This parasite has been reported to cause colony deterioration in combination with other bee mites in Pakistan [141–143]. The *Tropilaelaps* spp. mites are native to Asia and feed on the hemolymph of developing honey *A. dorsata* brood [139]. *T. clareae* has been reported in Pakistan, especially during springtime and it shows infestation of the brood in about 8% and only 0.4% of adult workers [144]. Another parasite, the greater wax moth (*Galleria mellonella*) is a natural pest of *A. mellifera* colonies. This parasite has been reported in Pakistan and is normally found during the hotter season (May–November), with a peak abundance of 14.8 ± 3.9 moth larvae/hive [145].

Viruses

About 20 different viruses cause diseases in honey bees [146]. The most common of them are deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV), IAPV, Kashmir bee virus (KBV), chronic bee paralysis virus (CBPV) and slow bee paralysis virus (SBPV) [147]. The isolation of these viruses has not been reported from Pakistan, however, there is a high chance that some viruses are circulating in the honeybee colonies in the country [148] because many of these viruses have been reported in the neighboring countries e.g. Iran [149], India [150] and China [133].

The wild and isolated honey bees have less diversity of viral infections, and they are mostly reported in the managed colonies of both *A. cerana* and *A. mellifera* [133].

Colony collapse disorder (CCD)

This complex phenomenon involves the abnormal abandonment of the colony by the majority of worker bees, leaving behind the queen and only a few nurse bees. While the exact cause of CCD remains unknown, it is believed that a combination of various chemical stressors (such as pesticides, heavy metals, airborne particulate matter), biological threats (including mites and viruses), malnutrition, and insufficient foraging habitats may interact additively or synergistically, ultimately leading to CCD [48, 93, 136] (Fig. 3).

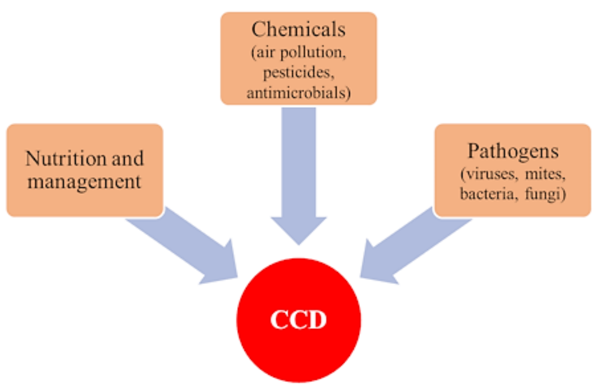

Fig. 3 Potential causes of Colony Collapse Disorder (CCD) in honey bees.

Electromagnetic signals

Modern electromagnetic pollution is pervasive, affecting virtually every corner of the planet with various forms of electromagnetic signals. Several reports have reported that different types of electromagnetic waves, even at very low intensities, can impact the health of honey bees and other wildlife [151, 152]. Erdoğan & Cengiz [153] studied the application of electromagnetic field (EMF) and electric field (EF) on the honey bee foraging in an experimental area. The results showed that with increasing electromagnetic radiation and electric field strength, the foraging frequency and foraging time decreased. These effects occur due to impaired cognitive and motor abilities of adult honey bees exposed to EMF even at extremely low frequencies [154,155]. Migdał et al. [156] reported that the honey bees exposed to EF at 50 Hz and variable intensities showed a lower number of occurrences of walking, self-grooming, and contacts between individuals than the control bees and had significantly higher protease activity compared to the control group. Electromagnetic radiations also decline the colony strength of honeybees [10]. Li et al. [157] reported that the exposure of *A. cerana* larvae to extremely low-frequency EMF reduced their survival rate and development. Research in Pakistan has also reported the effect of electromagnetic pollution on human, animal, and plant health [158-161], however, the investigations regarding honey bees are not yet available.

Conclusions

The significance of honey bees in Pakistan's agro-economy and vegetative biodiversity cannot be overstated. Honey bees play a crucial role in agricultural production as pollinators and offer valuable products. Furthermore, apiculture presents a significant opportunity to bolster managed honey bee populations and enhance the production of products. The majority of the challenges to the honey bee population and diversity are similar to those faced by these insects in neighboring countries. However, some factors are more prominent in Pakistan, for example, climate change and deforestation. Apiculture, itself is mainly promoting Western honey bee culture which is a threat to other managed honey bee biodiversity in Pakistan. Moreover, the rising level of environmental pollution is causing population and diversity decline in both the managed and feral honey bees. Many biological hazards have crossed the species barriers, transferring from exotic to local and *vice versa*, creating huge devastation by exposing naive populations to new pathogens. There exists a notable gap in research and data availability concerning apiculture and its influencing factors in Pakistan. For instance, obtaining a reliable figure on the total number of managed honey bee colonies in the country is challenging, as various sources provide wide-ranging estimates (ranging from 0.3 to 1.1 million). Similarly, data regarding the export and local consumption of products are scarce. Although honey bee pathogens are known to regularly impact apiculture, only a few studies have reported their presence in Pakistan. Additionally, information on the effects of antibiotics, microplastics, and electromagnetic signals on Pakistani honey bees is not accessible through scientific web search engines.

It is imperative for all stakeholders in apiculture, including beekeepers and researchers, to collaborate and establish associations to share reliable information on honey bee health. Furthermore, crossdisciplinary collaborations, such as adopting a one health, one hive approach, are essential for sharing knowledge and tools to address honey bee health challenges, as these hazards, such as antimicrobial-resistant pathogens and agrochemicals, pose risks to human and animal health as well. Additionally, honey bees serve as models for numerous research studies and are utilized as indicators for monitoring environmental health. Government bodies and policymakers should make informed decisions regarding the protection of honey bee health. This could involve implementing regulations on pesticide handling, including marketing, storage, and application, to mitigate risks to honey bee health.

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Conflict of interest

The authors have declared that no competing interests exist.

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