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Review Paper

Colony Collapse Disorder of honey bee: A neoteric ruction in global apiculture

ABSTRACT

For last few years, honey bee disappearance by destruction of the entire colony attracted the attention of many researchers where they used the term colony collapse disorder (CCD), but, they failed to explain the exact reason of this phenomenon. Many schools of thoughts are there where several causes like *Varroa* mite, honey bee tracheal mite, fungal pathogen *Nosema*, neonicotinoid group of pesticides, Israely Acute Paralysis Virus, migratory bee keeping, genetic factors, and parasitic phorid fly etc. play the key role either singly or in combinations. Investigations are solely needed to pin down a causal relationship among CCD and aforesaid factors those hitherto been considered the case.

Keywords: *CCD, honeybee, parasites, pathogens, neonicotinoids*

1. INTRODUCTION

Honeybees are obviously the most valuable crop pollinators [1] to agriculture because they can be easily transported to pollinator-dependent crops [2]. Pollinators support the reproduction of nearly 85% of the world's flowering plants [3] and 35% of global crop production [4]. In recent years, however, managed honeybees have been increasingly suffering from various diseases, pesticides and other environmental stresses. Accordingly, the contributions of wild pollinators to crop pollination (comprised of many other bee species as well as other insects) appear to have been increasing in relevance [5,6]. Explained as well as unexplained reasons of honey bee colony loss have been reporting for several decades [2,7]. However, normal and catastrophic losses of honey bee colonies reported from several parts of the world [8], the magnitude and speed of recent hive losses appear unprecedented [9,10]. The main causal suspects have been assumed to be parasitic mites [11], fungal infection [12], viral diseases [13] and interaction among them [2,14]. While, viral and microsporidian infections have been linked to enhance mortality and declining health in bee colonies [14,15], surveys have not directly addressed behavioural changes. Honey bees are very much prone to attack by different biotic stresses like fungus, bacteria, viruses, protozoa and ecto-parasitic mites [16]. Infection from these pathogens can be fatal to honey bees, but parasitic mites *Varroa destructor* and *Acarapis woody* appear to be the most harmful to colonies overall [17]. However, as a new pathogen agent, *Acarapis woodi* have been imported into Asia with the introduction of the European bee. Viruses have been spread by *Apis mellifera* beekeepers migrating or shipping bees to new areas and infecting and sometimes decimating *Apis cerana* colonies. Several viruses like deformed wing virus, Israely Acute Paralysis Virus are also responsible for colony loss of honey bee worldwide. Many other organisms like *Nosema* also attacks on honey bees[12] and cause heavy death of them. Hence, it can be argued that honey bee colony loss or honey bee death or colony collapse disorder (CCD) is one of the most important and burning issue in the recent period globally for both the apiculturists, environmentalists and many other related scientists.

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2. COLONY COLLAPSE DISORDER

Apiculture has been in decline globally over recent decades, as is shown by the decreasing numbers of managed honey bee (*Apis mellifera* L.) colonies [18,19]. Apart from socio-economic factors, which can only be addressed by politicians, sudden losses of honey bee colonies have occurred, and have received considerable public attention [11]. Indeed, in the last few years, the world's press has been full of eye catching but often uninformative headlines proclaiming the dramatic demise of the honey bee, a world pollinator crisis and the spectre of mass human starvation. "Colony Collapse Disorder" (CCD) in the USA has attracted great attention, and scientists there and in Asia and Europe are working hard to provide explanations for these extensive colony losses [11,20]. CCD is the phenomenon that occurs when the majority of worker bees in a colony disappear and leave behind a queen, plenty of food and a few nurse bees to care for the remaining immature bees and the queen [21,22]. Once thought to pose a major long term threat to bees, reported cases of CCD have declined substantially over the last few years. While such disappearances have occurred throughout the history of apiculture and were known by various names (disappearing disease, spring dwindle, May disease, autumn collapse, and fall dwindle disease), the syndrome was renamed colony collapse disorder in late 2006 in conjunction with a drastic rise in the number of disappearances of western honey bee (*Apis mellifera*) colonies in North America [7]. But hives cannot sustain themselves without worker bees and would eventually die. This combination of events resulting in the loss of a bee colony has been called Colony Collapse Disorder. There have been many theories about the cause of CCD, but the researchers who are leading the effort to find out why are now focused on these factors.

2.1 *Varroa* mite

The ecto-parasitic mite *Varroa destructor* [17] is considered as one of the most serious pests of beehives, causing huge losses to honey bees (*Apis mellifera* L) and great economic loss to the beekeeping industry [23]. Parasitism can result in a loss of up to 25% of adult weight, severe deformations of the wings and reduced longevity of worker and drone honey bees [24,25]. Ecto-parasitic mite of honeybee *Varroa destructor* was first described by Oudemans [26] from Java on *Apis cerana.* In 1962- 63, the mite was found on *Apis mellifera* in Hong Kong and Philippines [27] and spread rapidly from there. This mite parasitizes only honeybees. The specialized mouthparts enable them to feed on bee brood and adult honeybees by sucking haemolymph. The mite brood development is closely synchronized with bee brood development and colonies heavily infected by *Varroa* produce little or no honey [28]. Colonies infested with *Varroa destructor* have significantly reduced worker bee populations and eventually die if left without controlling. The development of infested brood is also affected because emerged bees have a low weight and shorter life span [24].
V. destructor mite has been associated with *A. cerana* in Sub-continent Pak-India for the last thousands of years and became a serious pest of *A. mellifera* [29]. It is an invasive species from Asia [30,31] and a dominant factor for honey bee colony losses. It negatively affects honey bee health and immune system, thus causing physical and physiological deterioration [17]. Out of 17-18 mitochondrial haplotypes of *V. destructor,* Korean (K) haplotype (a haplotype is a set of DNA variations, or polymorphisms that tend to be inherited together) is the most virulent [32]. It also serves as a vector for several harmful positive-strand RNA viruses like Deformed Wing Virus which translate into severe disease complex leading to higher mortality in bees, lowered productivity, reduced honey production and a decrease in pollination efficiency [33]. Without periodic treatment, most of the honey bee colonies in temperate climates would collapse within a period of 2–3 years [30]. The *Varroa* mite has been a threat to world beekeeping industry and now a potential threat to Indian apiculture [34]. In India, it has ravaged *A. mellifera* colonies from northern states showing infestation in 90% apiaries [35].

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2.2 Tracheal mite

Acarapis woodi distributed widely where honey bees are found [36,37]. It infests the tracheal system of adult bees, queens, workers and drones, which are all equally susceptible to its attack. Since it was first reported in *Apis mellifera* colonies in Europe in 1919, opinions regarding the extent of the damage it can cause to honey bee colonies have varied [38]. Life cycle takes place in the breathing tubes of honey bee. The mated female mite enters the breathing tubes through the first spiracle in the thorax [39,40], attracted to it by the puffs of air produced by the respiratory system of the bee [39]. After hatching of eggs, the nymphs and adults pierce the breathing tube wall with their mouthparts and suck the haemolymph and interfere with oxygen exchange in the breathing tubes [39]. Their mating also takes place within the breathing tubes where mite develops [41,42]. They spread quickly within the hive as a result of direct contact between bees. And by means of drifting of infested bees, infestation spread from one hive to another [43].

Typical visible symptoms of infestation are a large number of dead bees in the winter and there is enough honey in the hive, 'K' type wing condition [42] followed by Isle of Wight disease [44]. Indeed, it has been demonstrated that bees severely infested with the mite can forage normally [43,45]. Nevertheless, some differences exist with regard to the over-wintering capability of infested and healthy colonies. Infestation shortens the lifespan of the individual bees, so that severe infestation of colonies causes them to lose strength and thus increases a colony's susceptibility to winter losses [41]. In case of heavy infestation colonies die or have a dwindling population in late winter and spring. Mite can only be seen under microscope by opening the main trachea of thorax of suspected bees and all the stages *viz*. egg, nymph and adult can be seen at one time in a single trachea [46]. *Acarapis woody* is one of the major concerns for honey bee colony loss in India besides many countries of the world [47]. In India this mite causes a significant economic loss in many states where commercial apiculture drastically hampered [48,49].

2.3 Fungal pathogen, *Nosema*

Microsporidia are a highly derived group of fungi [50] that are obligate intracellular parasites of many animal species, particularly insects [51]. *Nosema* disease (Nosemosis), the original causative organism of which was identified as the unicellular microsporidium *Nosema apis* exactly 100 years ago [52], is considered to be one of the most prevalent and economically damaging of diseases of the western honey bee, *Apis mellifera*, and much information has accumulated on its biology, site of infection (ventricular cells of adult bees) and its impact on individual bees and colonies [53,54]. Yet it often goes unnoticed because *N. apis*, like all microsporidia, is microscopic in size and invisible to the naked eye and because *N. apis* rarely leads to the death of a diseased colony. Matheson [55] reported the widespread distribution of *N. apis* across the natural and introduced world range of *A. mellifera*, and DNA sequence data support the view that this microsporidium was indeed the sole causative agent of *Nosema* disease in the western honey bee up to the 1990s [50].

Analysis of *Nosema* isolates from *A. mellifera* from across the world [50], interrogation of DNA data bank entries and published records (based on rRNA sequence data) indicated that, post-2003, *N. ceranae* was widespread, and already found in North and South America, across Europe and Asia. It has been subsequently detected across Canada and USA [56,57] and has been confirmed in Central America [58], Australia [59] and North Africa [15]. In India occurrence of *Nosema* as one of the major reasons of honey bee colony loss by culminating huge economic damage has been reported [60,61].

Since its emergence as a novel pathogen of *A. mellifera*, *N. ceranae* has been generally associated with heavily diseased and moribund colonies [62]. For example, the first report of *N. ceranae* in European *A. mellifera* attributed heavy winter 2004-2005 colony losses in Spain to this EID (Emergent Invasive Disease) [63]. Unfortunately, there is no reliable field diagnostic symptom enabling a diseased worker bee to be identified without killing it, nor can the beekeeper recognize an infected queen [15,63]. The detailed metagenomic survey of CCD affected colonies of *A. mellifera* in the USA [64] recognised *N. ceranae* as a potential causative agent of CCD but statistically ruled it out as the primary agent responsible for CCD [65]. Experiments on caged worker bees have nevertheless

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revealed *N. ceranae* to be a potentially highly virulent pathogen [66], one that seems to be more pathogenic than *Nosema apis* [67]. In addition, *N. ceranae* places additional nutritional stress on individual bees [68,69], which may lead to riskier foraging and greater mortality of forager bees away from the hive. These effects of *N. ceranae* on the nutritional stress of individual worker bees may be exacerbated by a shortage of resources (pollen and nectar) [70], providing a mechanistic model for CCD.

2.4 Pesticide poisoning

Studying colonies of honeybees (*Apis mellifera* L.), researchers found that two widely-used pesticides [71] and acaricides [72] were directly responsible for the hive abandonment and death of several colonies. Neonicotinoids are used to control crop and ornamental plant pests such as aphids or leaf beetles, structural pests like termites and pests of domesticated animals such as fleas. Six neonicotinoid insecticides are used on crops: imidacloprid, clothianidin, thiamethoxam, dinotefuran, acetamiprid and thiacloprid showed varying degree of susceptibility against honey bees (Table 1). Imidacloprid was the first neonicotinoid on the world market, and is the most commonly used [73]. Neonicotinoids paralyze insects by blocking a specific chemical pathway that transmits nerve impulse in the insect's central nervous system [74]. These can be applied as seed coatings, soil drenches or granules, foliar sprays, by direct injection into tree trunks, or by chemigation (addition of the insecticide to irrigation water). This variety of application methods, along with their systemic properties and lower toxicity to vertebrates, is one of the primary reasons why these chemicals are increasingly used for crop protection [73]. An advantage of neonicotinoids for pest control is that their methods of application (i.e., a range of methods other than spraying) help to reduce direct contact to non-target insects during treatment. However, because these chemicals are systemic and absorbed into plant tissues, insects that rely on nectar, pollen, or other floral resources have increased oral exposure to residues of neonicotinoids or their metabolites (Figure 1). Residues have been recorded in pollen [75,76], nectar [77], and to a much lesser degree, other plant exudates [78].

Table 1. Toxicity of different neonicotinoid insecticides to honey bees, *Apis mellifera*

Neonicotinoid	Level	Known toxicity to honey bees (*Apis mellifera*)	
		Contact LD_{50}	Oral LD_{50}
Acetamiprid	M	7.1 µg/ bee – 8.09 µg/ bee	8.85 – 14.52 µg/ bee
Clothianidin	H	0.022 µg/ bee – 0.044 µg/ bee	0.00379 µg/ bee
Dinotefuran	H	0.024 µg/ bee – 0.061 µg/ bee	0.0076 – 0.023 µg/ bee
Imidacloprid	H	0.0179 µg/ bee – 0.243 µg/ bee	0.0037 – 0.081 µg/ bee
Thiacloprid	M	14.6 µg/ bee – 38.83 µg/ bee	8.51 – 17.3 µg/ bee
Thiamethoxam	H	0.024 µg/ bee – 0.029 µg/ bee	0.005 µg/ bee

Source: Iwasa *et al*.[103]; Nauen *et al*.[104]; Schmuck *et al*.[105]
H = Highly toxic; M = Moderately toxic
Toxicity: Highly toxic: LD_{50} < 2 µg/ bee; Moderately toxic: LD_{50} 2 – 10.99 µg/ bee; Slightly toxic: LD_{50} 11 – 100 µg/ bee; Practically non toxic: LD_{50} > 100 µg/ bee

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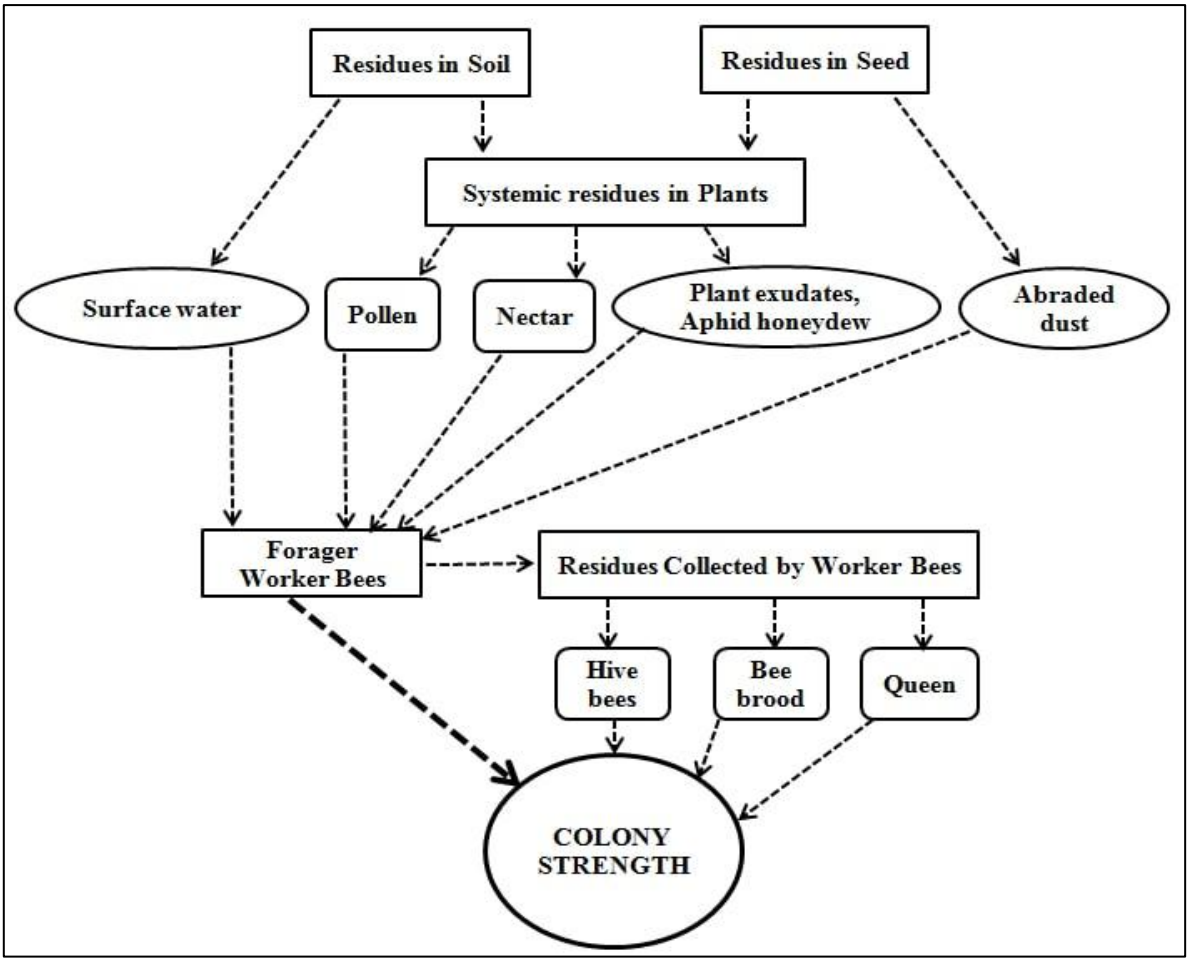

Source: Fischer and Moriarty [106]

Fig. 1. Routes of exposure of systemic pesticides to honey bee colonies

The failure of foraging bees to return to their hives has led many people to suggest that a link exists between CCD and the behavioral disruption observed with sub-lethal exposure to neonicotinoid insecticides [79]. As of yet, no single insecticide or combination of insecticides have been linked to CCD, though many chemicals have been found in hives [80]. Researchers that compared gene expression in honeybees from healthy colonies and from collapsed colonies found no link between expression of genes that code for proteins associated with the detoxification of insecticides and collapsed colonies [81]. This suggests that insecticide exposure, whether to neonicotinoids or another class, is not a primary factor in CCD. However, insecticide exposure may interact with other factors such as viruses or parasites to weaken colony health and increase susceptibility to CCD (Figure 2). Recent studies have demonstrated the combined effects of infection by honey bee gut parasites (*Nosema apis* and *N. ceranae*) and sub-lethal levels of neonicotinoids [82]. Alaux *et al*. [83] found that when they occurred together, imidacloprid, *N. apis*, and *N. ceranae* increased mortality more than neonicotinoid exposure or *Nosema* infection alone and reduced the ability to sterilize food. A reduced ability to sterilize stored food could make colonies more susceptible to other pathogens. *Nosema* infection may actually increase bee exposure to imidacloprid by causing energetic stress that leads to bees consuming more tainted nectar [83].

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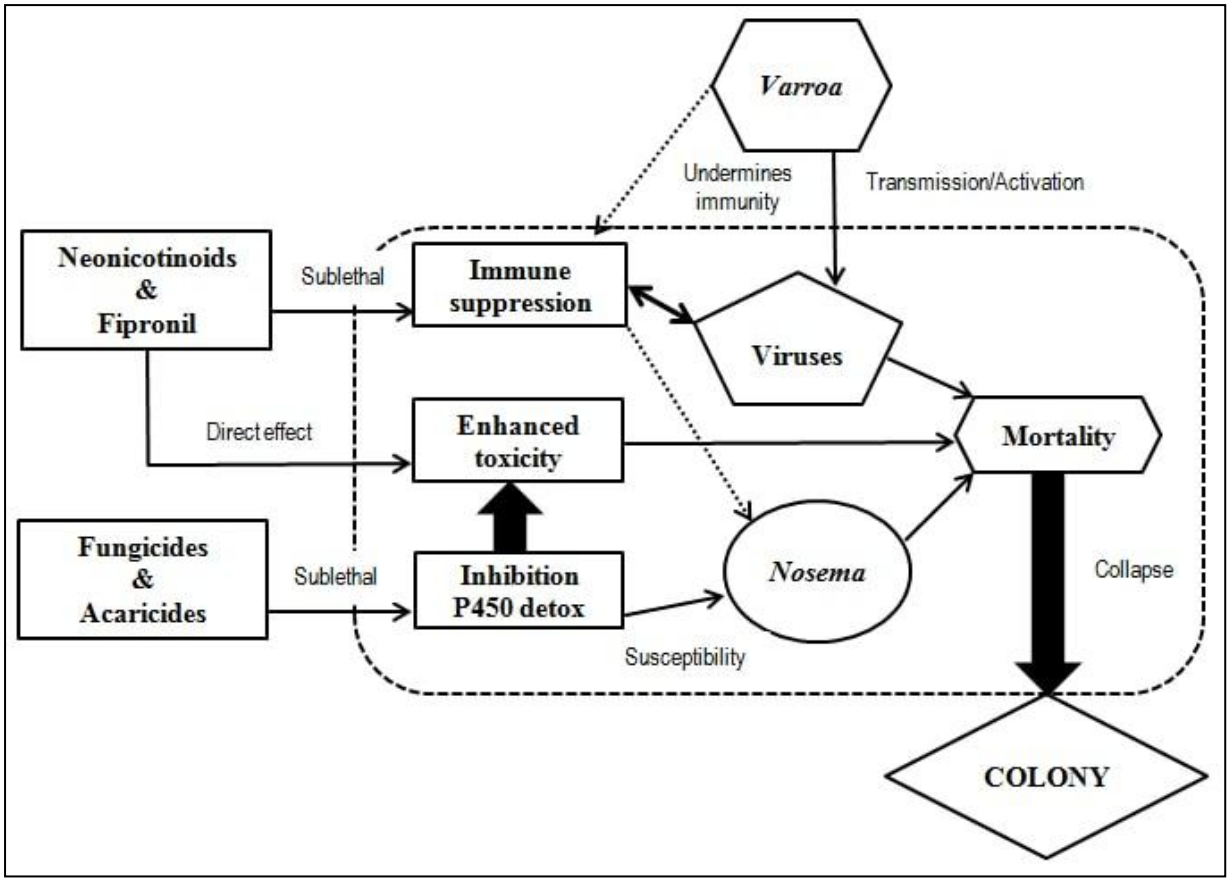

Source: Sanchez-Bayo *et al*. [82]

Fig. 2: Combined effect of pesticides and living parasites in CCD of honey bee

2.5 Migratory bee keeping

Management style is a broad category but it can include the type of income pursued with bees (honey production, pollination services, etc.) or what routine colony management beekeepers perform (splitting hives, swarm control, chemical use, etc.). Both of these vary considerably among beekeepers so this possible cause of CCD is given less attention. That said poor management can make any colony malady worse [84]. Researchers are concerned that trucking colonies around the country to pollinate crops, where they intermingle with other bees from all over, helps spread viruses and mites among colonies [19]. Additionally, such continuous movement and re-settlement is considered by some a strain and disruption for the entire hive [19], possibly rendering it less resistant to all sorts of systemic disorder [85].

2.6 Israeli Acute Paralysis Virus

In 2004, Israeli acute paralysis virus (IAPV) was discovered in Israel and at one time it was considered the cause of CCD [86]. It was named after the place it was first identified; its place of origin is unknown. In September 2007, results of a large-scale statistical RNA sequencing study of afflicted and unaffiliated colonies were reported. RNA from all organisms in a colony was sequenced and compared with sequence databases to detect the presence of pathogens. All colonies were found to be infected with numerous pathogens, but only the IAPV virus showed a significant association with CCD [86]; the virus was found in 25 of the 30 tested CCD colonies, and only in one of the 21 tested non-CCD colonies [14]. Research in 2009 has found that an indicator for an impaired protein production is common among all bees affected by CCD, a pattern consistent with IAPV infection [87]. It is conjectured that Dicistroyiridae, like the IAPV, cause degradation of the ribosome, which is responsible for protein production of cells, and that this reduced ribosomal function weakens the bees, making them more vulnerable to factors that might not otherwise be lethal [14]. Infected bees

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displayed shivering wings and crawling, disorientation, and progressed to paralysis and death within or outside the hive [13].

2.7 Parasitic phorid fly

In 2012, a parasitic fly (*Apocephalus borealis*) larva, known to prey on bumble bees and wasps, was found in a test tube containing a dead honey bee believed to have been affected by CCD [8] possibly indicating the phorid fly may be one cause of CCD [88]. The mature fly lays eggs in the bee's abdomen, which feed on the bee after hatching. Infected bees behave abnormally like zombie, foraging at night and gathering around lights like moths. Eventually the bee leaves the colony to die. The phorid fly larvae then emerge from the junction of head and thorax of the bee [8].
Another phorid fly *Megaselia rufipes*, found as a potential facultative parasitoid of honey bee in Italy [89] and several records of parasitisation of this species on living insect specimen have already reported by many scientists; hence it can also be considered as a cause of honey bee mortality and colony weakness under tropical and temperate regions.

2.8 Electromagnetic radiation

A study on the non-thermal effects of radio frequency (RF) on honey bees (*Apis mellifera* L.) reported there were no changes in behaviour due to RF exposure from DECT cordless phone base stations operating at 1,880–1,900 MHz, however, a later study established that close-range electromagnetic field (EMF) may reduce the ability of bees to return to their hive. In April 2007, news of this study appeared in various media outlets, beginning with an article. Though cellular phones were implicated by other media reports at the time, they were not covered in the study. A review of 919 peer-reviewed scientific studies investigating the effects of EMF on wildlife, humans and plants included seven studies involving honey bees; six of these reported negative effects from exposure to EMF radiation, but none specifically demonstrated any link to CCD. The review noted that according to one study when active mobile phones were kept inside beehives, worker bees stopped coming to the hives after 10 days [90]. The same study also found drastic decrease in the egg production of queen bees in these colonies and stated "electromagnetic radiation exposure provides a better explanation for Colony Collapse Disorder (CCD) than other theories". The review authors concluded: "existing literature shows that the EMRs are interfering with the biological systems in more ways than one" and recommended recognising EMF as a pollutant [91].

3. REVIEW OUTLOOK

Currently available global data and knowledge on the decline of pollinators are not sufficiently conclusive to demonstrate that there is a worldwide pollinator and related crop production crisis. Although honey bee hives have globally increased close to 45% during the last 50 years; declines have been reported in individual countries like Austria [92], Bulgaria [93], Canada [94], Croatia [95], Denmark [62], England [96], France [71], Greece [97], Italy [98], Netherlands [99], Norway [33], Poland [100], Scotland [101], Switzerland [102], USA [18,21] and India [22]. However, human activities and their environmental impacts may be detrimental to some species but beneficial to others, with sometimes subtle and counter-intuitive causal linkages. Pollination is not just a free service but one that requires investment and stewardship to protect and sustain it. Scientists, beekeepers, government officials, various industries, etc. are beginning to investigate various avenues of CCD. These groups have initiated a number of investigations into the possible causes and at this point, it is safe to assume that most potential causes of CCD are being investigated. There should be a renewed focus on the study, conservation and even management of native pollinators particularly honey bees to complement the managed colony tradition by suppressing CCD.

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