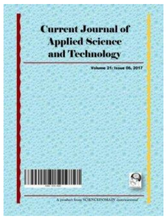

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Colony Collapse Disorder of Honey Bee: A Neoteric Ruction in Global Apiculture

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Authors' contributions

This work was carried out in collaboration with all authors. Author DR designed the study matter and wrote the major portion of manuscript. Authors PD and DM collected the literature. Author PKS managed the entire text of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

For last few years, honey bee disappearance by the 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, Israeli 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. The study was undertaken to make a detailed idea on CCD, its impact, probable causes, economic importance, controversy etc. by assembling the inferences of a number of global researchers.

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1. INTRODUCTION

Honeybees are obviously the most valuable crop pollinators [1] to agriculture because they can be easily transported to pollinator-dependent crops [2]. Both wild and managed pollinators support the reproduction of nearly 85% of the world's flowering plants [3] where western honey bee, eastern honey bee, some bumble bee and stingless bee significantly contribute as managed pollinators according to crop and location [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 for 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]. 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 ectoparasitic 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, Israeli Acute Paralysis Virus are also responsible for colony loss of honey bee worldwide. Many other organisms like *Nosema* also attacks honey bees and cause the massive 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 issues in the recent period globally for both the apiculturists, environmentalists and many other related scientists. Despite many current research efforts on these phenomena, no any single driver has yet fixed the definite cause. Since, apiculture is one of the major fields of agriculture and allied sciences, honey bee colony losses become a major discussable issue in last few years. Moreover, in order to integrate different schools of thoughts in a compact column for the convenience of researchers, readers, apiculturists etc. this topic has been reviewed.

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 happens when the majority of worker honey bees in an existing colony suddenly disappear by leaving behind a queen, adequate amount of food and a few nurse bees in order to care for the next time [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. Similar disappearances have taken place throughout the history of global apiculture and were recognised by different names *viz.* disappearing disease, spring dwindle, May disease, Autumn collapse, fall dwindle disease etc. Later on, the syndrome was renamed colony collapse disorder among the people in late 2006 in North America with the disappearances of western honey bee (*Apis mellifera*) colonies [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 opinions about the cause of CCD like several biotic stresses, abiotic factors and/or their cumulative effects, but the researchers who are leading the effort to find out why are now focused on these parameters.

2.1 *Varroa* Mite

The ectoparasitic 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 adult weight, severe deformations of the wings [24] and reduced longevity of worker and drone honey bees [25]. The ectoparasitic 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 the 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 control. The development of infested brood is also affected because emerged bees have a low weight and shorter lifespan due to viral infection [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]. *A. mellifera* colonies showed infestation about 90% apiaries in India, 25% in Japan, 30% in the USA, 2-50% in Europe and 10-80% in the Middle East [35].

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]. The 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]. According to the previous report, this mite caused a significant economic loss in many states of India where commercial apiculture drastically hampered [48]. Gradually, it has successfully invaded Asia, Europe, parts of Africa, North and South America except Australia, New Zeeland, Norway and Sweden [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, are 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 databank 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 in 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]. This parasite is now widespread throughout the world and gained capacity to infect other Hymenopteran different from honey bees but *A. mellifera* found to be most susceptible [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 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 with 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 (Fig. 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} (µg/ bee)	Oral LD_{50} (µg/ bee)
Acetamiprid	M	7.1– 8.09	8.85 – 14.52
Clothianidin	H	0.022– 0.044	0.00379
Dinotefuran	H	0.024– 0.061	0.0076 – 0.023
Imidacloprid	H	0.0179– 0.243	0.0037 – 0.081
Thiacloprid	M	14.6– 38.83	8.51 – 17.3
Thiamethoxam	H	0.024– 0.029	0.005

Source: Iwasa et al. [111]; Nauen et al. [112]; Schmuck et al. [113]
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

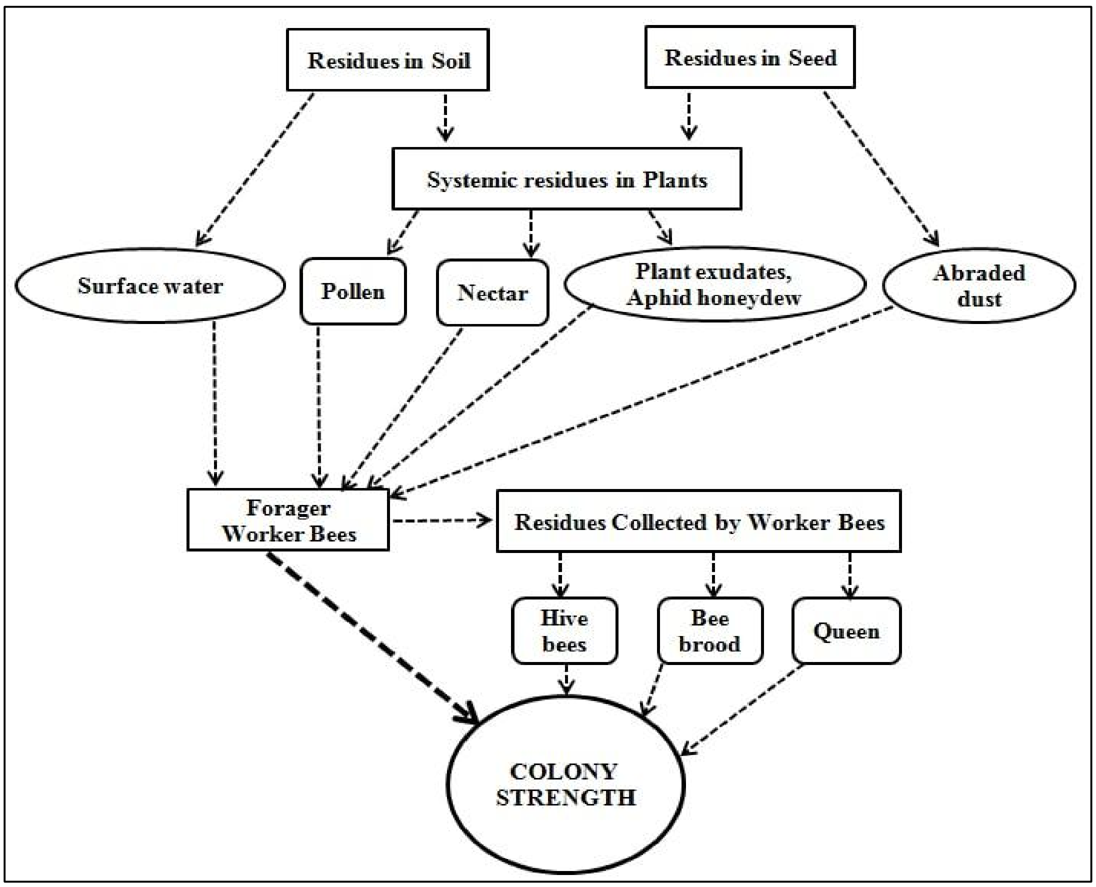

Fig. 1. Routes of exposure to systemic pesticides to honey bee colonies
Source: Fischer and Moriarty [114]

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 (Fig. 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].

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 could make any colony malady worse [84]. Researchers are concerned that infestation of healthy colonies by various viruses and mites spread from another infested population may be a resultant effect of trucking colonies around the country to pollinate crops [19]. Moreover, such continuous movement from one place to another and re-settlement of colonies is considered for disruption of the entire hive [19], leads to its poor resistance against all sorts of systemic disorders [85].

2.6 Israeli Acute Paralysis Virus

In 2004, Israeli acute paralysis virus (IAPV), an RNA virus was discovered in Israel and at one time it was considered the prime cause of CCD [86]. The news drew an international attention of virus infection in honey bees followed by in September 2007, results of a large-scale statistical RNA sequencing study were reported. But, the role of IAPB in triggering honey bee colony losses alone or in conjugation with other,

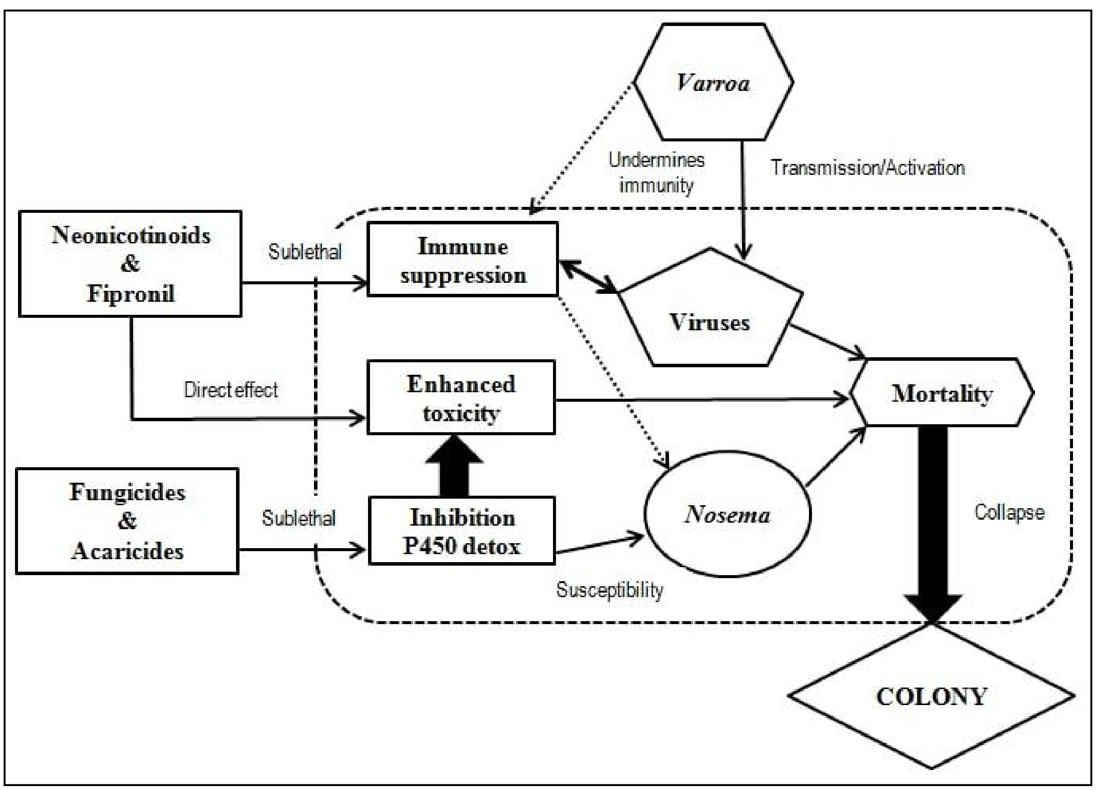

Fig. 2. Combined effect of pesticides and living parasites in CCD of honey bee
Source: Sanchez-Bayo et al. [82]

still remains a research priority. In a research, it has been found that in spite of numerous pathogens infection, only the IAPV virus showed a significant association with CCD [14] with the evidence of its presence in 83% tested CCD colonies, and only in one of the 21 tested non-CCD colonies [86]. A research in 2009 documented that an indicator for an impaired protein production is common among all CCD affected bees which determines a pattern consistent with IAPV infection [87]. In 2010 it has been concluded in a research that establishment of IAPB takes place as a persistent infection in honey bee populations and the phenotypic pathology of this virus differs due to standing genetic variations among different strains [88]. The family Dicistroviridae, closely related to IAPV [89,90], cause degradation of the ribosome of honey bees, which is responsible for protein production of cells and thus making them more vulnerable to factors that might not otherwise be lethal [88]. Infected bees displayed shivering wings and crawling, disorientation, and progressed to paralysis and death within or outside the hive [13].

2.7 Parasitic Phorid Fly

A parasitic fly (*Apocephalus borealis*) larva, previously known to prey on bumble bees and wasps, was found to infesting honey bees, believed to be a cause of CCD [8,91]. The mature female lays eggs in the bee's abdomen, which feed on the internal anatomy of bee after hatching. Infected bees behave abnormally like zombie, foraging at night and gathering around a nearby light source like nocturnal Lepidopterans followed by leaves the colony to die. A pile of dead honey bees were a common symptom of this incidence beneath that light in the very next morning. The matured phorid fly larvae then emerge from the junction of head and thorax of the dead bees [8].

Another phorid fly *Megaselia rufipes*, found as a potential facultative parasitoid of honey bee in Italy [92] 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 in tropical and temperate regions.

2.8 Electromagnetic Radiation

A study conducted in Punjab, India 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 [93], however, a later study established that close-range electromagnetic field (EMF) may reduce the ability of bees to return to their hive [94]. In April 2007, news of this study appeared in various media outlets, beginning with an article [95]. Though cellular phones were implicated by other media reports at the time, they were not covered in the study [95]. 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 [96]. 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 [97].

3. REVIEW OUTLOOK

Currently available global data and knowledge on the decline of honey bees are not sufficiently conclusive to demonstrate that there are a worldwide pollinator and related crop production crisis. Although honey bee hives have globally increased close to 45% during the last 50 years [98]; declines have been reported in individual countries like Austria [99], Brazil [100], Bulgaria [101], Canada [102], Croatia [103], Denmark [62], England [104], France [71], Greece [105], Italy [106], Netherlands [107], Norway [33], Poland [108], Scotland [109], Switzerland [110], 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.

4. CONCLUSION

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 honey bees to complement the managed colony tradition by suppressing CCD.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Spivak M, Mader E, Vaughan M, Euliss NH. The plight of the bees. Environ Sci Technol. 2010;45:34-38.
2. Williams GR, Tarpy DR, vanEngelsdorp D, Chauzat M, Cox-Foster DL, Delaplane KS, Neumann P, Pettis JS, Rogers REL, Shutler D. Colony collapse disorder in context. Bioessays. 2010;32:845–46.
3. Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? Oikos. 2011;120:321-26.
4. IPBES. Summary for policymakers of the assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services on pollinators, pollination and food production. SG Potts, VL Imperatriz-Fonseca, HT Ngo, JC Biesmeijer, TD Breeze, LV Dicks, LA Garibaldi, R Hill, J Settele, AJ Vanbergen, MA Aizen, SA Cunningham, C Eardley, BM Freitas, N Gallai, PG Kevan, A Kovács-Hostyánszki, PK Kwapong, J Li, X Li, DJ Martins, G Nates-Parra, JS Pettis, R Rader, BF Viana (eds.). Secretariat of the intergovernmental science-policy platform on biodiversity and ecosystem services, Bonn, Germany. 2016;36.
5. Kremen C, Miles A. Ecosystem services in biologically diversified versus conventional farming systems: Benefits, externalities, and trade-offs. Ecol Soc. 2012;17:58-62.
6. Garibaldi LA, Aizen MA, Klein AM, Cunningham SA, Harder LD. Global growth and stability of agricultural yield decrease with pollinator dependence. Proc Natl Acdm Sci. 2011;108:5909-14.
7. Oldroyd BP. What's killing American honey bees? PLoS Biol. 2007;5:e168.
8. Core A, Runckel C, Ivers J, Quock C, Siapno T, Denault S, Brown B, DeRisi J, Smith CD, Hafernik J. A New Threat to Honey Bees, the Parasitic Phorid Fly *Apocephalus borealis*. PLoS ONE. 2012; 7:e29639.
9. vanEngelsdorp D, Hayes Jr J, Underwood RM, Pettis JS. A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. J Apicul Res. 2010; 49:7-14.
10. Ratnieks LWF, Carreck NL. Clarity on honey bee collapse. Sci. 2010;327:152–53.
11. Neumann P, Carreck NL. Honey bee colony losses. J Apicul Res. 2010;49:1-6.
12. Ellis JD, Munn PA. The worldwide health status of honey bees. Bee World. 2005; 86:88-101.
13. Maori E, Lavi S, Mozes-Koch R, Gantman Y, Peretz Y, Edelbaum O, Tanne E, Sela I. Isolation and characterization of Israeli Acute Paralysis Virus, a dicistro virus affecting honeybees in Israel: Evidence for diversity due to intra- and inter-species recombination. J General Virol. 2007;88: 3428–38.
14. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan PL, Briese T, Hornig M, Geiser DM, Martinson V, vanEngelsdorp D, Kalkstein AL, Drysdale A, Hui J, Zhai J, Cui L, Hutchinson SK, Simons JF, Egholm M, Pettis JS, Lipkin WI. A metagenomic survey of microbes in honey bee Colony Collapse Disorder. Sci. 2007;318:283-287.
15. Higes M, Martin-Hernandez R, Garrido-Bailon E, Gonzalez -Porto AV, Garcia-Palencia P, Meana A, Nozal MJD, Mayo R, Bernal JL. Honey bee colony collapse due to *Nosema ceranae* in professional apiaries. Environ Microbiol Reports. 2009; 1:110-13.
16. Booton RD, Iwasa Y, Marshall JAR, Childs DZ. Stress-mediated Allee effects can cause the sudden collapse of honey bee colonies. J Theorel Biol. 2017;420:213-219.
17. Anderson DL, Trueman JWH. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol. 2000;24: 165-89.
18. Ellis JD, Evans JD, Pettis JS. Colony losses, managed colony population decline and Colony Collapse Disorder in the United States. J Apicul Res. 2010;49:134-36.
19. Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones HR, Neumann P, Settele J. Declines of managed honey bees and

beekeepers in Europe. J Apicul Res. 2010; 49:15-22.
20. vanEngelsdorp D, Meixner MD. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Inverteb Pathol. 2010;103:80-95.
21. vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood RM, Tarpy DR, Pettis JS. Colony Collapse Disorder: A descriptive study. PloS ONE. 2010;4:e6481.
22. Sharma NS. Study of impact of insecticides on declining honeybee colonies at Jamner district: Jalgaon (Maharashtra) India. Indian J Sci Res. 2014;9:111-16.
23. Al-Abbadi A, Nazer IK. Control of *Varroa* mite (*Varroa destructor*) on honey bees by aromatic oils and plant materials. J Agril Mar Sci. 2003;8:15-20.
24. Skubnik K, Novacek J, Fuzik T, Pridal A, Paxton RJ, Plevka P. Structure of deformed wing virus, a major honey bee pathogen. PNAS. 2017;114:3210-3215.
25. Organtini LJ, Shingler KL, Ashley RE, Capaldi EA, Durrani K, Dryden KA, Makhov AM, Conway JF, Pizzorno MC, Hafenstein S. Honey bee deformed wing virus structures reveal that conformational changes accompany genome release. J Virol. 2017;91:e01795-16.
26. Oudemans AC. On a new genus and species of parasitic acari (*Varroa*) notes. Leyd Mus. 1904;24:216-22.
27. Delfindo MD. Mites of the honeybee in Southeast-Asia. J Apicul Res. 1963;2:13-14.
28. Ritter W. *Varroa* disease of honeybee *Apis mellifera*. Bee world. 1981;62:141-53.
29. Sihag RC. Incidence of *Varroa*, *Euvarroa* and *Tropilaelaps* mites in the colonies of honey bee, *Apis mellifera* L. in Haryana (India). Amer Bee J. 1988;128:212-213.
30. Underwood RM, Currie RW. Effect of concentration and exposure time on treatment efficacy against *Varroa* Mites (Acari: Varroidae) during indoor winter fumigation of honey bees (Hymenoptera: Apidae) with formic acid. J Econ Entomol. 2005;98:1802-09.
31. Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of *Varroa destructor*. J Invertebr Pathol. 2010;103:96-119.
32. Benjamin D, Evans JD, Chen YP, Gauthier L, Neumanna P. Dead or Alive: Deformed Wing Virus and *Varroa destructor* reduce the Life Span of Winter Honeybees. Appl Environ Microbiol. 2012;78:4981-87.
33. Dahle B. The role of *Varroa destructor* for honey bee colony losses in Norway. J Apicul Res. 2010;49:124-25.
34. Gatoria GS, Chhuneja PK, Singh J, Blossom K. *Varroa* mite-A potential threat to the Indian apiculture. Indian Bee J. 2004;66:112-22.
35. Brettell LE, Martin SJ. Oldest *Varroa* tolerant honey bee population provides insight into the origins of the global decline of honey bees. Scientific Reports. 2017;7: 45953.
36. Eckert JE. *Acarapis* mites of the honey bee, *Apis mellifera* L. J Insect Pathol. 1961;3:409-25.
37. Shaw FR, Fischang WJ, Balboni ER, Eversole JW. External mites on honeybees in U.S. Glean Bee Cult. 1961;89:402-03.
38. Rennie J, White PB, Harvey EJ. Isle of Wight Disease in hive bees. Trans Royal Soc Edinburgh. 1921;52:737-79.
39. Eischen FA. Overwintering performance of honey bee colonies heavily infested with *Acarapis woodi* (Rennie). Apidologie. 1987;18:293–303.
40. Danka RG, Villa JD, Rinderer TE, Delatte GT. Field test of *Acarapis woodi* (Acari: Tarsonemidae) infestation and of colony production by four stocks of honey bees (Hymenoptera: Apidae). J Econ Entomol. 1995;88:584–91.
41. Otis GW, Scott-Dupree CD. Effects of *Acarapis woodi* on overwintered colonies of honey bees (Hymenoptera: Apidae) in New York. J Econ Entomol. 1992;85:40–46.
42. Danka RG, Villa JD. A survey of tracheal mite resistance levels in US commercial queen breeder colonies. Am Bee J. 2000; 140:405–07.
43. Perepelova LP. Acarine disease in USSR (Russia). Bee World. 1927;8:133–34.
44. Bailey L. The Isle of Wight Disease. Central Association of Bee-Keepers, Poole, UK. 2002;11.
45. Williams KR, Sugden EA, Webster TC. Effects of honey bee queen type and age on tracheal mite infestation in Kentucky. Am Bee J. 1994;134:838.
46. De Jong D, Morse RA, Eickwort GC. Mite pests of honey bees. Ann Rev Entomol. 1982;27:229-52.
47. Singh S, Makkar GS, Chhuneja PK. Record of parasitic mites associated with

different species of honey bees in India. Insect Env. 2013;19:37-42.

48. Kaushik HD. In: Mites, their Identification and Management. Ed. Yadav PR, Chauhan R, Putatunda BN, Chhillar BS. CCS, HAU, Hisar. 2001;86-87.
49. Shakib V, Mehdi ES. Unprecedented first record of infestation level *Acarapis woodi* (Rennie) and overwintering ability in Savojbolagh regions of Alborz province in Iran. J Entomol Zool Stud. 2016;4:1-13.
50. Lee J, Besana AM, Genersch E, Gisder S, Nanetti A, Tam DQ, Chinh TX, Puerta F, Ruz JM, Kryger P, Message D, Hatjina F, Korpela S, Fries I, Paxton RJ. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. J Invrtebr Pathol. 2007;96:1-10.
51. Larsson R. Ultra structure, function, and classification of Microsporidia. Prog Protistol. 1986;1:325-90.
52. Zander E. Tierische Parasiten als Krankheitserreger bei der Biene. Munchener Bienenzeitung. 1909;31:196-204.
53. Fries I. *Nosema apis*, sampling techniques and honey yield. J Apicul Res. 1984;23: 102-05.
54. Fries I. *Nosema apis* - a parasite in the honey bee colony. Bee World. 1993;74:5-19.
55. Matheson A. World bee health report. Bee World. 1993;74:176-212.
56. Chen Y, Evans JD, Smith IB, Pettis JS. *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. J Invrtebr Pathol. 2008;97: 186-88.
57. Williams GR, Shafer AB, Rogers RE, Shutler D, Stewart DT. First detection of *Nosema ceranae*, a microsporidian parasite of European honey bees (*Apis mellifera*), in Canada and central USA. J Inverteb Pathol. 2008;97:189-92.
58. Calderon RA, Sanchez LA, Yanez O, Fallas N. Presence of *Nosema ceranae* in Africanized honey bee colonies in Costa Rica. J Apicul Res. 2008;47:328-29.
59. Giersch T, Berg T, Galea F, Hornitzky M. *Nosema ceranae* infects honey bees (*Apis mellifera*) and contaminates honey in Australia. Apidologie. 2009;40:117-23.
60. Dar SA, Ahmad SB. Occurrence of nosemosis (*Nosema apis)* affecting honey bee (*Apis mellifera L.)* colonies in Kashmir. Appl Biol Res. 2013;15:53-56.
61. Ansari MJ, Al-Ghamdi A, Adgaba N, Khan KA, Alattal Y. Geographical distribution and molecular detection of *Nosema ceranae* from indigenous honey bees of Saudi Arabia. Saudi J Biol Sci. 2017;24: 983-991.
62. Vejsnaes F, Kryger P. Factors involved in the recent increase in colony losses in Denmark. J Apicul Res. 2010;49:109-10.
63. Higes M, Martin R, Meana A. *Nosema ceranae*, a new microsporidian parasite in honey bees in Europe. J Invrtebr Pathol. 2006;92:93-95.
64. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan PL, Briese T, Hornig M, Geiser DM, Martinson V, vanEngelsdorp D, Kalkstein AL, Drysdale A, Hui J, Zhai JH, Cui LW, Hutchison SK, Simons JF, Egholm M, Pettis JS, Lipkin WI. A metagenomic survey of microbes in honey bee colony collapse disorder. Sci. 2007;318:283-87.
65. Paxton RJ. Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)? J Apicul Res. 2010;49:80-84.
66. Higes M, Garcia-Palencia P, Martin-Hernandez R, Meana A. Experimental infection of *Apis mellifera* honey bees with *Nosema ceranae* (Microsporidia). J Invrtebr Pathol. 2007;94:211-17.
67. Paxton RJ, Klee J, Korpela S, Fries I. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie. 2007;38:558-65.
68. Mayack C, Naug D. Energetic stress in the honey bee *Apis mellifera* from *Nosema ceranae* infection. J Invrtebr Pathol. 2009; 100:185-88.
69. Naug D, Gibbs A. Behavioural changes mediated by hunger in honey bees infected with *Nosema ceranae*. Apidologie. 2009; 40:595-99.
70. Naug D. Nutritional stress due to habitat loss may explain recent honey bee colony collapses. Biol Conserv. 2009;142:2369-72.
71. Chauzat MP, Martel AC, Blanchard P, Clement MC, Schurr F, Lair C, Ribiere M, Wallner K, Rosenkranz P, Faucon JP. A case report of a honey bee colony poisoning incident in France. J Apicul Res. 2010;49:113-15.

72. Harz M, Muller F, Rademacher E. Organic acids: Acute toxicity on *Apis mellifera* and recovery in the haemolymph. J Apicul Res. 2010;49:95-96.
73. Elbert A, Haas M, Springer B, Thielert W, Nauen R, Applied aspects of neonicotinoid uses in crop protection. Pest Mngt Sci. 2008;64:1099-1105.
74. Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Ann Rev Entomol. 2003;48: 339-64.
75. Laurent FM, Rathahao E. Distribution of imidacloprid in sunflowers following seed treatments. Agril Food Chem. 2003;51: 8005-10.
76. Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME. Quantification of imidacloprid uptake in maize crops. J Agril Food Chem. 2005;53: 5336-41.
77. Krischik VA, Landmark AL, Heimpel GE. Soil-applied imidacloprid is translocated to nectar and kills nectar feeding *Anagyrus pseucocci* (Girault) (Hymenoptera: Encyrtidae). Environ Entomol. 2007;36: 1238-45.
78. Girolami V, Mazzon L, Squatini A, Mori N, Marzaro M, Dibernardo A, Greatti M, Giorio C, Tapparo A. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. J Econ Entomol. 2009;102:1808-15.
79. Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, Padovani E, Porrini C, Sabatini AG. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. J Apicul Res. 2010;49:52-59.
80. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS ONE. 2010;5: e9754.
81. Johnson RM, Ellis MD, Mullin CA, Frazier M. Pesticides and honey bee toxicity-USA. Apidologie. 2010;41:312-31.
82. Sanchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N. Are bee diseases linked to pesticides? – A brief review. Environ Intl. 2016;89-90:7-11.
83. Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environ Microbiol. 2010; 12:774–82.
84. Meixner MD, Costa C, Kryger P, Hatjina F, Bouga M, Ivanova E, Buchler R. Conserving diversity and vitality for honey bee breeding. J Apicul Res. 2010;49:85-92.
85. Barrionuevo A. Bees vanish; Scientists race for reasons. New York Times, 24 April. 2007;1.
86. Hunter W, Ellis J, vanEngelsdorp D, Hayes J, Westervelt D, Glick E, Willams M, Sela I, Maori E, Pettis J, Cox-Foster D, Paldi N. Large-scale field application of RNAi technology reducing Israeli Acute Paralysis Virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae). PLoS Pathog. 2010;6:e1001160.
87. Maori E, Paldi N, Shafir S, Kalev H, Tsur E, Glick E, Sela I. IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion. Insect Mol Biol. 2009;18:55-60.
88. Chen YP, Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, Visscher PK, DeGrandi-Hoffman G, Bonchristani H, Zhao Y, vanEnglesdorp D, Delaplane K, Solter L, Evans JD. Israeli Acute Paralysis Virus: Epidemiology, Pathogenesis and Implications for honey bee health. Plos Pathog. 2010;10:e1004261.
89. Mullapudi E, Fuzik T, Pridal A, Plevka P. Cryo-electron microscopy study of the genome release of the Dicistrovirus Israeli Acute Bee Paralysis Virus. J Virol. 2017; 91:e02060-16.
90. Mullapudi E, Pridal A, Palkova L, de Miranda JR, Plevka P. Virion structure of Israeli Acute Bee Paralysis Virus. J Virol. 2016;90:8150-8159.
91. Khattab MM, Nowar EE. The first records of the parasite zombie fly (*Apocephalus borealis* Brues) on honeybee, *Apis mellifera* in Egypt. Int J Agril Sci Res. 2014;4:37-42.
92. Dutto M, Ferrazzi P. *Megaselia rufipes* (Diptera: Phoridae): A new cause of facultative parasitoidism in *Apis mellifera*. J Apicul Res. 2014;53:141-45.
93. Kumar NR, Sangwan S, Badotra P. Exposure to cell phone radiations

produces biochemical changes in worker honey bee. Toxicol Int. 2011;18:70-72.
94. Favre D. Mobile phone induced honey bee worker piping. Apidologie. 2011;42:270-279.
95. Kimmel S, Kuhn J, Harst W, Hermann S. Effects of electromagnetic exposition on the behaviour of honey bee (*Apis mellifera*). Environ Sys Res. 2007;8: 1-8.
96. Sahib SS. Impact of mobile phones on the density of honey bees. Mun Entomol Zool. 2011;6:396-99.
97. Sharma VP, Kumar NR. Changes in honey bee behaviour and biology under the influence of cellphone radiation. Curr Sci. 2010;98:1376-78.
98. Traynor KS, Rennich K, Forsgren E, Rose R, Pettis J, Kunkel G, Medella S, Evans J, Lopez D, vanEngelsdorp D. Multiyear survey targeting disease incidence in US honey bees. Apidologie. 2016;47:325-347.
99. Brodschneider R, Moosbeckhofer R, Crailsheim K. Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol. J Apicul Res. 2010;49:23-30.
100. Pires CSS, Pereira FM, Lopes MTR, Nocelli RCF, Malaspina O, Pettis JS, Teixeira EW. Weakness and collapse of bee colonies in Brazil: are there cases of CCD? Pesq Agropec Bras Brasilia. 51: 422-442.
101. Ivanova EN, Petrov PP. Regional differences in honey bee winter losses in Bulgaria during the period 2006-9. J Apicul Res. 2010;49:102-03.
102. Currie RW, Pernal SF, Guzman-Novoa E. Honey bee colony losses in Canada. J Apicul Res. 2010;49:104-06.
103. Gajger IT, Tomljanovic Z, Petrinec Z. Monitoring health status of Croatian honey bee colonies and possible reasons for winter losses. J Apicul Res. 2010;49: 107-08.
104. Aston D. Honey bee winter loss survey for England, 2007-8. J Apicul Res. 2010;49: 111-12.
105. Hatjina F, Bouga M, Karatasou A, Kontothanasi A, Cha-Ristos L, Emmanouil C, Emmanouil N, Maistros AD. Data on honey bee losses in Greece: A preliminary note. J Apicul Res. 2010;49:116-18.
106. Mutinelli F, Costa C, Lodesani M, Baggio A, Medrzycki P, Formato G, Porrini C. Honey bee colony losses in Italy. J Apicul Res. 2010;49:119-20.
107. Van Der Zee R. Colony losses in the Netherlands. J Apicul Res. 2010;49:121-23.
108. Topolska G, Gajda A, Pohorecka K, Bober A, Kasprzak S, Skubida M, Semkiw P. Winter colony losses in Poland. J Apicul Res. 2010;49:126-28.
109. Gray A, Peterson M, Teale A. An update on recent colony losses in Scotland from a sample survey covering during 2006-2008. J Apicul Res. 2010;49:129-31.
110. Charriere JD, Neumann P. Surveys to estimate colony losses in Switzerland. J Apicul Res. 2010;49:132-33.
111. Iwasa T, Motoyama N, Ambrose JT, Roe RM. Mechanism for the differential toxicity of neonicotinoid insecticides in honey bee, *Apis mellifera*. Crop Prot. 2004;23:371-78.
112. Nauen R, Ebbinghaus-Kintscher U, Schmuck R. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Mngt Sci. 2001;57:577-86.
113. Schmuck R, Schoning R, Stork A, Schramel O. Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Mngt Sci. 2001;57:225-38.
114. Fischer D, Moriarty T. Pesticide risk assessment for pollinators. In: summary of a SETAC Pellston Workshop, Pensacola, Florida, USA. 2011;15.

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