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Pests, parasitoids, and predators: Can they degrade the sociality of a honeybee colony, and be assessed via acoustically monitored systems?

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Abstract
Honey bees are one the world's most important contributors to plant pollination. Their unexplained decline has become a matter of great concern. Potential factors destroying bee colonies include pathogens, pests, pesticides and environmental changes. The lethal effects of these factors have been extensively observed, but elucidating the mechanisms that may cause the collapse of an entire colony has been largely neglected. In this review, along with lethal effects we address the nonlethal effects of insect invaders (like insect pests, parasitoids and predators) and mites on social behavior of in-hive honeybee colonies. We also describe the acoustic emissions produced during confrontations with insect invaders. Then, we briefly describe multiple in-hive monitoring systems, and propose our own simple sound collection system which can prove as a useful tool to upgrade previously developed in-hive monitoring systems. Lastly, based on existing knowledge, we present hypothetical strategies towards maintaining normal hive social behavior to mitigate the effects instigated by colony collapse disorder (CCD).

Keywords: Honey bees, beehive monitoring, insect invaders, mites, sociality, acoustics

1. Introduction

Honeybees and other animal pollinators are crucial to the preservation of ecosystems, as well as sustainable crop production and global food security. They account for 75% of all plant based food production [1]. Recently, pollinator bees have been the matter of environmental debates, as they have been facing a population decline. This decline first received major attention in the year 2006 when an enormous number of honeybee colonies died from a phenomenon since named "Colony Collapse Disorder (CCD)" [2].

Apiarists have been reporting CCD for many years. Due to this phenomenon, they experience great economic losses resulting from reduction in honey and beeswax production, as well as insufficient bees for pollination. The population starts to diminish and then there is a rapid disappearance of bees, sometimes within just few days. There is a growing consensus in the field that there are multiple forces working together to weaken the bee immune system [3, 4].

Many factors contribute to the decline of honeybee population in a colony, such as nutritional imbalance, pesticide related death, genetic variability and more importantly, disease, pests, and predation [5]. Bee diseases are present throughout the globe and are responsible for large annual losses in bees, honey and equipment; and moreover, add greatly to the cost of production. Various bacterial, fungal and viral diseases responsible for CCD have been reported. Such disease related colony loss endangers pollination, human health, and are projected to cause $100 billion in agricultural loss [6, 7, 8].

In addition to disease, pesticides and genetic variability, other key factors have a role in CCD and threaten honeybee populations; these include insect predators, pests, and parasitoids. Yellow jacket wasps, hornet [9], crab spider [10], ants, wax moth, black bear and most prominently humans are stated as important predators of the bee colony [5]. The most damaging group of pests are predatory vertebrates that desire to feed on honey, such as amphibians, reptiles, birds and mammals [11].

Most studies have been intensively described about the lethal effects of pest and predators on honeybee sociality, but their non-lethal effects have been rarely documented in literature. Sociality, in actual, is a kind of survival rejoinder against the evolutionary stress [12]. There is a key effect of sociality on threats encountered by social insects, and this effect is in lowering the active population sizes, and boosting up the population genetic subdivision.

But the key effect of sociality on threat initiated from social insects is through its involvement in the ecological success against pronounced danger (i.e. invasive species) to native biota [13]. During several circumstances, the prompt dispersal of invasive species of social insects has indisputably been due to some reasons that make invasive organisms (relating to other taxa) much effective, the reasons included are absence of parasites, predators and some other opponents restraining their numerals in their local ranges [14, 15].

Sociality of honeybee owns a wide range of traits, the basic traits are: overlapping of adult generation, reproductive division of labor, and cooperative brood care [16]. Moreover, other imperative traits that in consequence make honeybees ecologically effective in their local ranges include: (1) their ability to take over resources via majority population [17, 18], (2) their competency of self-dispersion, prompt reproduction, and (3) more necessarily the hasty defense against predators, and in broad spectrum, behavioral as well as ecological plasticity that is legitimate by sociality [19].

Mostly pathogens, insect pests and parasites interfere the sociality of honeybee colony inside the beehives, but sociality respond these interferences in various ways, for instance, to reduce the risk of pathogenic infections the antimicrobial defenses [20], immune systems [21], multiple mating, as well as reductions in colony relatedness [22, 23] are persuaded. Other than the aforementioned social traits, grooming behavior by the worker bees to confiscate the ectoparasites (i.e. varroa mites) is also well described. This is very important strategy for maintaining their colony health [24, 25].

Conversely, to survive within host colonies, the Small Hive Beetles (SHBs) mimic trophallactic solicitation that is an alternative scheme to exploit the food interchange between nest-mates of honeybee colony [26, 27]. But the role of sound communications inside the nest in evolution of honeybee sociality has immense importance [28] because they produce distinctive sound upon disclosure to sub-lethal concentrations of several airborne toxicant, and stressors like predatory mites [29]. In the same way, when hornets are around the *Apis mellifera*, they produce sound (i.e. piping sound or hisses), also stated as shimmering [30, 31]. Qandour *et al.* [32] established some signals indicating that the honeybee fitness can be monitored and determined by using acoustic analysis.

Some invertebrates exhibit certain activities that interfere with sociality of the honeybees, for instance, hoverflies do not attack on honeybees but feed on pollens collected by bees, the form of feeding called as Kleptoparasitism [33]. It means that they are competitors of bees in obtaining pollen resources that ultimately disturb social nature of bees. Hoverflies not only morphologically resemble Hymenopterans but also have ability to produce same acoustic signals [34]. In special concern with sound communication, when honeybees confront insect predator, they yield hissing sound with a certain frequency level [30]. After going through above mentioned literature, it is supposed that honeybees and their non-lethal insect predators can employ this acoustic behavior as a defense strategy, and a scheme for gaining access to pollen resources. More notably, this acoustic conduct is thought to be used as an important tool to check the status of honeybee sociality.

Attempts to monitor activity of honeybees at hive entrance by automating the system started date back about a century [35]. Many latest researches have been undertaken to determine the decline of honeybees around the globe [36]. Various research projects tried to computerize the process of investigation for honeybees [37, 38, 39]. No doubt, few of these researches have made some development in automating the process but related researches are still ongoing and need further improvements. As an automated data acquired from beehive system may assist to lessen beehive losses by providing beekeepers with very useful and applicable data. A monitoring system (an automated) permits beekeepers to observe their beehives even from a distant place (remote monitoring), also permits beekeepers to pinpoint potential problems and address them, and most essentially, minimizes the need of manual routine examination of the hives that instigate stress among the honeybee colonies [40].

In this review, our main emphasis is to discuss some important insect invaders (i.e. predators, pests, and parasitoids), and their non-lethal effects that may disturb honeybee sociality. While, our other interest is to provide some information about how to explore advanced techniques for bee researches, particularly for the study of honeybee sociality. As due to any disturbance in honeybee sociality, the acoustic emissions from in-hive bee colony are produced therefore, we are hopeful that these emissions will also be produced due to insect invaders. Moreover, we expect that in-hive monitoring of these acoustic emissions by developed monitoring systems can assist beekeepers to get information about health status of their bee colonies.

2. Insect threats to in-hive honeybees

In addition to pathogens, there are number of insect pests and parasites that cause massive deterioration in apiaries at global level. Such pests and parasitoids, and their percent range of infestation is described below (Table 1).

Table 1: Damage caused by various pests/ parasitoids to honeybee colonies in different countries.

S. No	Pests/Parasitoids	Infestation level in bee colonies	Country	References
1	*Varroa destructor*	40-80%	Australia	Morawetz *et al.* [155]
2	*V. destructor*	55.9%	USA	Kulhanek *et al.* [156]
3	*Aethina tumida*	58.8%	USA	Kulhanek *et al.* [156]
4	*A. tumida*	85.36%	Nigeria	Lawrence and Neumann *et al.* [157]
5	*Galleria mellonella* L.	5-72%	India	Vijayakumar *et al.* [158]
6	*G. mellonella L*	78%	Pakistan	Sohail *et al.* [159]
7	*Apocephalus borealis*	12-38%	USA	Core *et al.* [65]
8	*Braula coeca*	1.2 ± 1.5/100 bees	South Africa	Straus *et al.* [160]
9	*B. coeca*	$\leq 0.5\%$	Libya	Alfallah and Mirwan [161]
10	*B. coeca*	0.45 ± 2.6/100 bees	Uganda	Chemurot and de Graaf [162]

2.1. Hive beetles are detrimental and threaten honeybee social behavior

Small hive beetles (*A. tumida/*or SHBs) are pests of economic importance that cause reduction in brood and honey production, in extreme circumstances they can also cause total destruction (Fig. 1) of honey bee colonies. Beside the detrimental impacts, SHBs also exhibit non-lethal impacts on honeybees to survive within colony. The SHBs mimic trophallactic solicitation that is an alternative scheme to exploit the food interchange between nest-mates of honeybee colony [26, 27]. During begging or suppliant, the SHBs relocate nearby the bee's mandibles, later touch them using its particular mouth parts anteriorly and proximally. Moreover, to touch bee's mandibles, SHBs also use their forelegs, which is much alike to the bee-bee behavior in trophallaxis [41, 42]. The observations shows that prior the feeding process, mouth parts of the SHB and the host bee come in contact which later serve for the food uptake. This might permit an adaptive selection of the SHB, for instance, to target bees (especially host honeybees) with minimum level of alarm pheromone, thereby may limit the opportunity to cause injury and instead escalate success [27]. That's why we can think trophallactic solicitation as an important non-lethal approach for SHBs to acquire food from host species (causing ultimate disturbance in honeybee brood care, an important trait of sociality). Apart from the trophallactic solicitation, these beetles also persuade some other non-lethal impacts to apiary, for example, they form eating tunnels and damage the cell caps in honey combs [43], while larvae and faeces of these beetles alter both color and taste of the honey [44]. Formerly, it was believed that these beetles have severe impacts merely on weak colonies, but proof endorses that they also have capability to cause momentous impairment to strong colonies. On the other hand, these beetles also act as a scavenger of destabilized colonies and referred to status of secondary pest [6, 45, 46, 47].

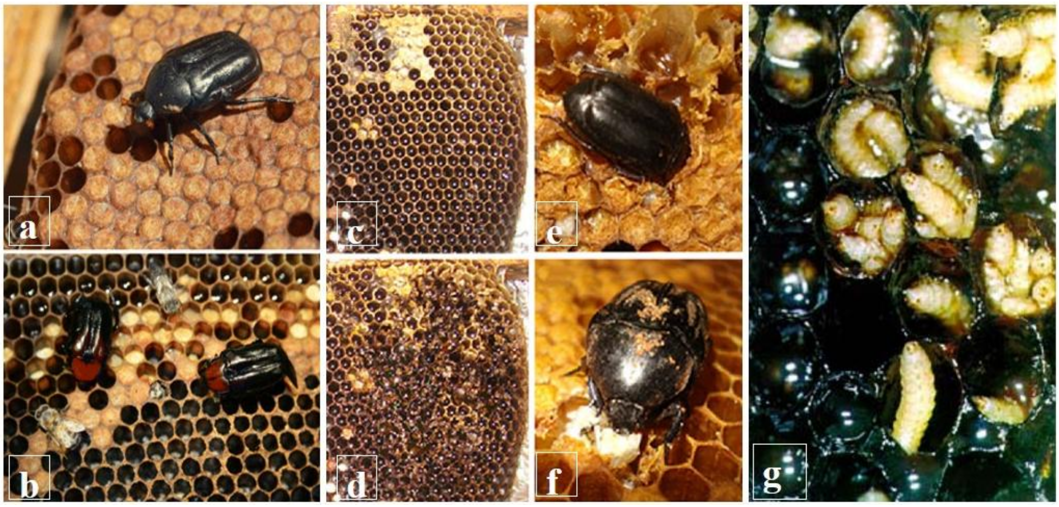

Fig 1: Species of adult large African beetles, and larvae of small hive beetle (*Aethina tumida*). (a) *Oplostomus fuligineus* (Photo credit: B. Oldroyd). (b) *O. haroldi* (Photo credit: M. Allsopp). (c-d) Sections of frame exhibiting damage to opened honey cells due to African beetles (Photo credit: doi:10.1007/s13592-012-0149-6). (e-f) Brood feeding by African beetles via searching deep into comb and on the comb surface respectively (Photo credit: doi:10.1007/s13592-012-0149-6). (g) *A. tumida* larvae feeding on honey inside the comb cells (Photo credit: Jeff Lotz).

Not only the SHBs, the two African hive beetles (*Oplostomus haroldi* and *Oplostomus fuligineus* [Coleoptera, Scarabideae, Cetoniinae]) (Fig. 1) are also likely to become invasive species, which is extremely detrimental to beekeeping in the world. According to reports, both species prey upon *Apis mellifera* brood, and are also studied as pests in southern and eastern Africa correspondingly [48, 49, 50]. So, activities performed by all the aforementioned beetles may induce collapse within bee colonies.

2.2. Do moths pose a real problem to bees?

No doubt, the greater wax moth (*Galleria mellonella* L.) and lesser wax moth (*Achroia grisella* Fabricius) thereof on apiaries are key apprehensions [51]. For instance, the *G. mellonella* are pervasive pests of different honeybee associated species (*A mellifera* Linnaeus and *A. cerana* Fabricius) and their tunneling larvae outpace large number of webs (Fig. 2) which result galleriasis and subsequent colonies to flee [52]. While *A. grisella* are secondary pests because they only cause nuisance, and a disorder "bald brood" to honeybee colonies [53] which in consequence make the products unsellable.

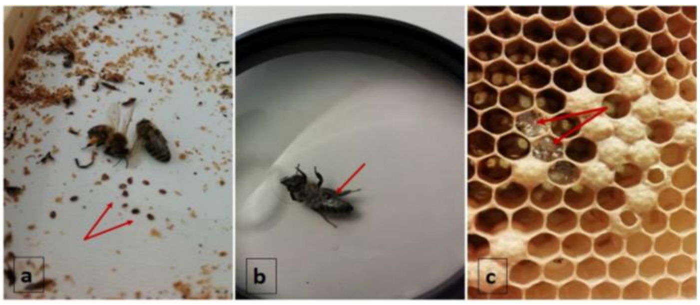

Fig 2: Pictures "a & b" both exhibit mites (*Varroa destructor*) infected honeybees collected from a hive while, picture "c" exhibit web formation inside the cells of honeybee comb due to wax moth infestation. The V. destructor, and moth developed webs are shown in the direction of arrows.

Wax moths are not much serious pests, and may occasionally cause problems when they invade in weak or stressed colonies [54, 55]. Until now, they don't pose a threat to honeybees, and we suspect they may become threats later. Other than the wax moths, the death's-head hawkmoths (*Acherontia atropes*) also merely create some disturbance in bee sociality by consuming their food storages (honey/nectar), but do not cause any serious/lethal threats to the colony population so do not have any economic importance [54]. One of the distinctive ability and tactic of *A. atropes* is to mimic the piping sound of the queen which avert them to be attacked by the worker bees in the colony [56]. We believe that this mimicking/or imitation may lead to worker bees being hindered in recognizing and attacking the invaders, thus undermining the sociality.

2.3. Do ants threaten bees and their colonies?

Ants are not frequently the pest of severe demolition to honey bee colonies but stated as wonderful invertebrate predators of terrestrial ecosystems [57]. Sometimes, however, certain species may go inside the colonies to explore food or to establish nesting sites. Ants normally found in pollen traps or between inner and outer shield of beehives, and their intrusion result to produce stereotyped behavior in the *A. mellifera* [58, 59]. The behavior of ants found in pollen traps to explore food, and the establishment of nesting sites within colonies may influence honeybee's social system (sociality).

Multiple ant species are documented that create problems for beekeeping but among all these the frequently considered species are weaver ant (*Oecophylla smaragdina*), the black ant (*Monomorium indicum*), *Monomorium destructor*, *Oligomyrmex* spp., *Dorylus* spp., the fire ants (*Solenopsis* spp.) and *Formica* spp. [6] and Argentine ants (*Iridomyrmax humilis* Mayr [60]. It is reported that there are many native or introduced African species of honeybees whose continued disturbance due to ants can cause bee colonies to abscond [54, 61]. This is an unrest to the social nature of the honeybee colony.

Argentine ants "*I. humilis* Mayr" are capable of destroying resilient and populous colonies, and are therefore known as serious pest of honeybees in South Africa [60]. These ants are also wreaking damage by vectoring the insect virus, which is designated as "Deformed Wing Virus (DWV)" and is involved in CCD [62]. Continuous fights by ant species persuade absconding in *A. mellifera* and *A. cerana* colonies. Several Poneroid ants including: *Eciton* sp., *Anomma* sp., and *Dorylus* sp., in assemblage of thousands, can destroy whole apiary merely in few hours [63]. On the other hand, *Camponotus compressus* F. (carpenter ant) was narrated occasionally a serious pest of bee colonies in India and fire ants in the Southeastern parts of United State of America. Relatively, there are few ants stealing honey or brood but the genuine and non-lethal problem is: they start nesting inside the warm and dry hives and troubling the beekeepers in colony inspection [64], and no any lethal consequences are reported.

2.4. Parasitic infection of the phorid fly and the response of honeybee social behavior

Phorid flies (*Apocephalus borealis*; Diptera, Phoridae) are parasites [65] that are reflected to be important death factors for the acclimatized population of apiary in Central America [66, 67]. The *A. borealis* (native to North America) has been reported to parasitize bumble bees, paper wasps, spiders, and beetles but not honeybees [68, 69]. But recently, it is documented that *A. borealis* also infects, kills, and may have capability to pose greater intimidation to North American's beekeeping [65, 70].

But in case to fight against parasites, social bees develop cooperative behaviors like social immunity that mainly helps reduce parasite contact and the rate of propagation within colonies [71, 72]. Honeybee colonies acquire antimicrobial substances from the plant resins, and perform spatial segregation (abridged contact with foragers having maximum chances of being exposed to parasites at flowers), both of these behaviors or strategies help them to control parasitic infections [73-75]. Some other behaviors to mitigate the parasitic infection are also evolved by bee colonies like, social fever (the behavior in which in-hive temperature increases by beating wing muscles with coordinated efforts, which is mortal for parasites but not for the bees [76]. In addition, one of the important mechanism of honeybee sociality called "polyandry" reduces the parasitic loads in social system of bees [77, 78] and upsurges the level of intra-colony genetic variability by producing distinct patrilines (i.e. groups of bees having shared father) with altered vulnerabilities to both parasites and pathogens, resulting potential hindrance in their spread within the colony [72].

2.5. Bee lice (*Braula coeca*) instigate interference in colony social behavior

The *B. coeca*, an interesting parasite of honeybees [79], has been found in different continents of the world including Europe, Middle East and South Africa [80], and reported to be introduced very first time in the United States via imported queen bees [81]. They exhibit variant non-lethal behaviors like, the immature larvae of *B. coeca* feed upon wax, pollen, honey, and later tunnel inside the honey capping of bee comb. On other hand, adults of this bee lice do not eat honey inside the honeybee comb's cells, but instead they acquire their food right from the mouth of the host bees and the nectars. Moreover, in case of severe invasion, a queen bee may have a louse population (on her body parts i.e. head, thorax, and abdomen) within the range of 35 to 40 in number [82, 83] and must be able to loose most of her food, which will certainly disrupt the bee's food supply to the brood and therefore pose an ultimate impact on sociality of bee colony.

3. Defensive strategies in honeybees to counter parasites

At individual to colony level, honeybee especially *A. mellifera* adopt different resistance mechanisms for their defense against parasites. One of the key resistance mechanism at colony level is swarming, as it minimizes the intensity and reproduction of the parasites (i.e. Ectoparasite, *Varroa destructor*) within colony. In the new nest location, the bee tolerance at the colony level is instigated via reproductive effectiveness and its type as generally worker brood is produced at first, where parasites have lower level of fitness [84]. Upon parasitic invasion, the bee colony is also reported to exhibit another kind of swarming, the non-reproductive or absconding. During the absconding mechanism, all the colony members including the queen swarm leave the bee brood and stores behind, and later establish new nesting site elsewhere. This is the most efficient and pronounced defense mechanism in African honeybee's sociality as they suddenly left the hive whenever there are unfavorable circumstances [85].

To mitigate the effects of parasites (especially mites), multiple vertebrates show a common strategy designated as "grooming", which is used to remove the ectoparasites [86]. Moreover, this strategy in honeybees as mechanism of resistance against parasitic mite *Varroa destructor* has also been described by the researchers [87, 88, 89]. Peng *et al.* [24] described that bees can remove mites from body by their own (autogrooming) or they may get help from their nestmates (allogrooming). In contrast, Bąk and Wilde [90] described that they did not find any allogrooming behavior in all the honeybees used in their experiment. But they stated that *Apis mellifera mellifera* shows strongest autogrooming to remove mites, and this removal varies in its effectiveness, time duration as well as frequency. Anyhow, such behavior in honeybees strengthen their defense against the invader species, and prevent degrading sociality of bee colony.

4. The role of sound production during parasitic infection in the hive

It has also been pointed out that in the case of parasite attacks (especially mites) in honeybee colony, the bee buzzes are produced. The buzzing patterns (sound/acoustic features) of such bees fluctuates with the environment of the bee colony and the severity of the infection [32, 91, 92]. During any warning situation (pests, parasites or predator attack), hissing sound (single pulse pattern) of about 300-3600 Hz is produced. While, to prepare the colony for swarming during period of disturbance, the scouts produce piping sound (single pulse pattern) of about 100-2000 Hz [32]. In addition, Ferrari *et al.* [93] stated that while getting parasitic infection (mainly mite infection), the changes in sound characteristics (frequency shift) of honeybee colonies occur in the frequency range of about 100-300 Hz to a higher frequency range of 500-600 Hz, which serves as a forecaster for swarming to decrease honey loss. The variations in sound feature due to pathogenic infection, and mite's attack have already stated in the literature to some extent, but such variations due to insect pests and parasitoid infections are still needed to be investigated.

5. Lethal and non-lethal impacts of predatory wasps on apiaries are disrupting social activity of honeybees

Wasp is the key predators as it has very important predation effect on bee pollinators [94, 95]. Wasps can destroy or enormously effect about 10% of beehives which cause a higher financial forfeiture. They can also decrease honey production by curtailing supplies of nectar and honeydew, and compel honeybees to stay at home to defend their hive from invading wasps [96]. Predatory wasp species, as a substitute of providing cells with pollen, they clasp prey and take it back to nurse larvae in their nests which demonstrates opportunistic scavenger behavior [97]. The following describe lethal, but mainly non-lethal effects of yellow jacket wasp and hornet species on honeybee sociality.

5.1. Yellow jacket wasps

Multiple species of yellow jacket wasps including: *Espuma pensylvanica* (western yellow jacket), *V. germanica* (German wasp) and *V. vulgaris* (common yellow jacket), are general pests at outside eating zones in variant parts of the United States. But all these species create troublesome to beekeepers by confronting honeybee colonies, moreover, by carrying off both the honeybees as well as honey. During the phase of late summer and autumn, when there is reduction in population of prey insect, scarcity of nectar, and water stressed condition, the yellow jackets can create challenges for honeybees firstly by stealing resources from colonies and secondly by predating adult bees. Higher degree of predation can cause failures in bee population and overall colony health. Moreover, continual predatory pressure can also be a key problem for weaker colonies and may finally lead to collapse of entire colony [98].
The *V. germanica* exhibits a conservation issue because it is an important consumer of invertebrate prey, but a potential cause of damage to merely local arthropod population. In addition, on the hive entrance, they demonstrate an adaptive scavenger behavior against *A. mellifera ligustica* species [99].
To combat this predator, honeybees recruit couple of nest-mates and compel to flee. Moreover, heat balling behavior is also observed on many occasions that execute deterrence against predators. It does not seem to disturb the patrolling of bee colonies. It was found that agonistic events (fighting interactions) assisted by other nest-mates are generally resilient, involving physical combat and prolonged attacks at the hive entrance [100]. To extend the relevant study, Pusceddu *et al.* [101] observed further honeybee predation events by *V. germanica*. These events exposed couple of cases which were involved in dismemberment and sequestration of their victims. As we know that to take over resources (especially food resources) via majority population [17, 18], and defense against predators [19] are important traits of social system of bees therefore, aforementioned literature describe wasp species a key troubling factor for honeybee sociality.

5.2. Hornets

Hornets are members of the wasp family "Vespidae" within the genus "*Vespa*", and ferocious predatory species that can lead to abrasion and absconding of bee colonies [102]. In concern with Asian hornet (*Vespa tropica*), Seeley *et al.* [103] described its role that can cause absconding of *Apis florea* colony (about 6000 bees) after merely three hours of aggressiveness. Almost each country in Asia documented hornets as a mutual threat to apiaries of *A. mellifea* and *A. cerana* species [6].
Sometimes, honey bees exhibit variant behaviors against predators to defend their colonies, for example, *A. cerana* exhibits the bee-carpet, the heat balling and the shimmering (i.e. abdominal shaking [104-107]. In addition, *A. mellifera* also exhibits the same behaviors: the bee-carpet and the heat balling (except shimmering) moreover, is less effective than *A. cerana* colonies [104, 106, 108, 109]. During the fight (especially in case of heat balling), Cyprian honeybees (*A. mellifera*) can tempt death of its predator (*V. orientalis*) through asphyxiation, the death due to suffocation/deprived of oxygen [110]. Recently, balling behavior in *A. mellifera* is video recorded against *V. velutina* in Europe, suggests its further consideration and also add that more defensive behavior occurs in those hives which stay alive for slightly longer period of time [111].

6. Acoustic emissions of hive during predator attacks

In insects, multiple sounds are produced in different perspective that have been pronounced as singing, buzzing, squeaking, piping, and hissing or shimmering [32, 112, 113]. It has been stated that acoustic emissions in honeybee colony can be used as a defense mechanism against the predator [114]. When there is any warning situation, hissing sound of about 300-3600 Hz is produced by the colony [32]. Furthermore, when *A. mellifera cypria* face insect predator like Oriental hornet (*V. orientalis*), they yield hissing sound with dominant frequency of 6 KHz [30]. Moreover, the results of Kawakita and Ichikawa

[163] propose that it is possible to discriminate the insect flight sounds from environmental sounds at high accuracy of more than 0.95 in precision and recall. Results also described that classifying bees on specie level is challenging (showing low accuracy; 0.7-0.9 in precision and recall) but hornet specie (*Vespa simillima Xanthoptera*) could be better classified with 1.00 in precision as well as accuracy. Aforementioned predator exhibited a comparatively low frequency (about 100 HZ) than other bees (showing 200 Hz) which can prove valuable for acoustic characterization of bees and predators (i.e. hornet, wasp). The consistent evidence of acoustic emissions from above literature suggest their employment to maintain sociology.

7. Ectoparasitic mites are a major threat to apiary

No doubt, the contribution of parasites from Class *Insecta* is considered, but the ectoparasitic mites (Fig. 2) from class Arachnida are major threat to health of honeybees as well as to their entire colonies [115, 116]. Sammataro *et al.* [117] reported three parasitic mite species that have economic status because of their key role in destruction of apiaries on global level. They described them naming as tracheal (e.g. *Acarapis woodi*), Varroa (e.g. *Varroa jacobsoni*), and Tropilaelaps (e.g. *Tropilaelaps clareae*) mites.

According to reports, invasion of certain ectoparasitic mite species including *Varroa jacobsoni, V. destructor* and *Tropilaelaps mercedesae* have negative impacts on bee colonies [118-120], and perform crucial role to deteriorate their social system. Initially, *V. jacobsoni* and *V. destructor* were only limited to its natural host, *A. cerana*, the Eastern honeybee, but later were shifted to other host, *A. mellifera*, the European honeybee [121, 122]. Except Australia, the *V. destructor* is present over the globe that feeds mainly on honeybee fat body tissues [123], also results abnormal brood development and brood mortality within bee colonies [124].

In contrast, *T. mercedesae* is currently limited to Asia but it has potential to escalate and establish across many countries due to the reason of honeybee's trade on global level [124]. The interesting thing about *V. destructor* and *T. mercedesae* is their shared characteristics including: (1) they have same strategies for reproduction [117] and (2) both are also the vectors of deformed wing virus (DWV) in bee colonies [119]. These mite species are responsible for abnormal brood development and brood mortality so held major responsible factor to degrade social system of bees.

One of the important and severe problem associated with varroa mite is its role in viral transmission to honeybees which persuade deadly diseases. When such mites start feeding on hemolymph of bees, they transfer viruses directly into the open system of circulation, which consequently stretches to each cells of the body parts. The viruses transmitted by varroa mites including Deformed wing virus (DWV), as well as Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israel acute paralysis virus (IAPV) are a complex of linked viruses, and however, are fatal in circumstances when colonies are severely infected [125]. The infestation of *V. destructor* results major colony losses, as mites nurture on developing larvae as well as adults, may kill adequate number of individual bees causing CCD [120], and DWV invasions by mites have intensely deleterious effects on foraging [126], that in fact are major set backs for honeybee colonies to perform their social activities.

Social immunity, a trait in eusocial colonies of insects, contribute to combat the parasites, but this trait remained unsuccessful for western honeybees (*A. mellifera*) facing incursion of the *V. destructor*. Undoubtedly, *V. destructor* is severe threat to *A. mellifera* globally, even then some isolates of bees are known to resist mites infection via natural selection and generally via suppressing mite reproduction (which are honeybee sociality traits), but its underlying procedures are still uncertain. It is also confirmed by the direct experiments that honeybee's ability of uncapping/ recapping can slow down the reproductive success without any harm to nest-mates. Moreover, results provide remarkable indication that honeybees can overwhelm threatening parasites with simple qualitative as well as quantitative adaptive changes in behavior [127, 128].

In a project, Seccomb [129] developed the recording of in-hive acoustics for detecting infections of honeybee associated pests "i.e. mites and diseases" under the Small Business Innovation Research (SBIR) awards provided by US Department of Agriculture. The projected work builds on initial confirmation that intensities of *V. destructor* infestation in hives can be assessed from the sounds produced by colony. Moreover, the objective of this project is to develop algorithms using statistical as well as artificial neural network (ANN) for the purpose of rapid sonographic investigations. According to Qandour *et al.* [32], when bees continue to get sick with parasitic infection, the sound spectra are produced within beehive that can be monitored by remote beehive monitoring system. To fight against varroa mites in bee brood, a device was also invented consisting one middle wall that can be placed inside the beehive (note that it offers brood cells for female bees). Moreover, it is also provided with electrical heating device and control device that run the heating process (in the range of 39 °C to 45 °C, but favorably 39 °C to 42 °C) and then maintain the temperature for the encoded period of time (for detail information, Brunner [130]). So, it is fact that innovative systems and devices can be better used to monitor, detect and combat the factors degrading sociality of bee colonies, and may assist beekeepers to take curative measures for managing their apiaries.

8. In-hive honeybee colony monitoring systems

Multiple techniques, devices and monitoring systems have been used for monitoring in-hive bee colonies, and with the passage of time such techniques, devices and monitoring systems were updated for better performance. Primary study about such techniques and devices, and development in monitoring system is described below.

8.1. Preliminary Studies about colony monitoring

In case to estimate honeybee's associated behaviors, audio signals and audio processing techniques have been employed [131-133]. Sounds produced during honeybees visual and physical interactions with predators can be analyzed by different software including Avisoft [134], Seewave [135] and Raven Pro Interactive Sound Analysis Software (Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, USA). Ferrari *et al.* [93] planned an acoustic technique to forecast the swarming phase, and labelling the sounds produced by bee colony. Multiple devices of commercial use have been recommended for monitoring bee sounds. A sound analyzer named as "Apidictor" was presented in the beginning [136]. Moreover, Rangel and Seeley [137] introduced a microphone in overall five small observation beehives (three frames), and after a month interval they used those microphones for detection of specific type of honeybee produced sound designated as "piping". Upon detection of piping at particular threshold rate, in case of three signals detected however in 30 seconds, they powered on their video equipment in concern with taking images that were later scrutinized for movement of bees.

8.2. Developed monitoring systems

As an upgraded version, some patented systems/devices including: honeybee acoustic recording and analysis system, condenser microphone [29], and Acoustic sensors [138] working for beehive health monitoring, were introduced. Later on, Qandour *et al.* [32] also described a monitoring system of beehive that has ability for remotely monitoring of pest detection within colony, and for this the system compares the acoustic fingerprints of a hive to the acoustic fingerprints of a recognized status. Substitute methods for recording the bee sounds inside the beehive has been presented over the times, but of all those, the novel and updated substitutes are ultra-sensitive accelerometer, 805M1 accelerometer, and radar microphone [139-141].

When any voracious insect species of wasp or hornets attack honeybee colony (e.g. *A. cerana japonica*), they shows defensive behavior of "heat balling" against them. During this behavior these species start vibrating their wing muscles to produce heat inside the ball [142, 143]. In addition to this, another activity of immense importance in honey bees is swarming [144] which produce vibration. Therefore, radar microphone and accelerometers are much valuable tools to detect vibration instigated by all these activities but notably for the balling activity as it happens as a result of predator intervention which is key content of our review.

Other than acoustic monitoring systems, the imaging system [145], video surveillance or beemon system [40], passive radio frequency identification (RFID) system [146, 147, 148, 149, 150, 151], low cost platform [152], and wireless sensor networks [153] have also been stated for video monitoring of in-hive honeybee colonies. It is supposed that if at certain circumstances insects do not produce acoustics then video monitoring systems will be of worth consideration.

9. Our proposed system for recording beehive acoustics as a diagnostic of hive health

To keep an eye on the health of honeybees, multiple devices have been put inside the beehives. Buzz box and Arnia, both are state of the art remote beehive's monitoring systems which are being used nowadays to provide important information (sound, temperature, humidity, etc.) to our phone, tablet or PC. If we just need to collect sound data from healthy and infested colony then in present review, we propose a new, easy and automatic system (Fig. 3) which can listen inner dynamics of the hive sound. This dynamics will provide indication of fitness (health) or state of the whole colony. Now the question is: how does this state of a colony is monitored or what is the principle of our proposed monitoring system? We are well aware that our android cell phones are powerful computer in our pockets and have many interesting applications, therefore can prove as a helping tool for sound collection. In this regard, we can download and install "CinixSoft Scheduled Voice Recorder or Smart Voice Recorder" applications (Fig. 4) on our android phones. Both applications can be easily found from Google Play Store. The interesting thing about recorders, especially CinixSoft Scheduled Recorder, is not only capable of recording (with max. recording capacity of 125 hrs.) and adjusting multiple audio recordings of our selected day, time and duration but also useful to record sound into any of three audio formats including wav, 3gp, and mp4 (Fig 4). Additionally, this system will offer couple of function i.e. Filter noise and Skip silence. For the purpose of acoustic recording, any efficient microphone (Models: ECM 3005, Monacor; Probe microphone; or omnidirectional microphones) connected with android phone will be inserted within the beehive for sound recordings but the important thing for placement is to get the microphone closer with the bees in such a way that it shouldn't be put within the mass of bees to prevent propolization.

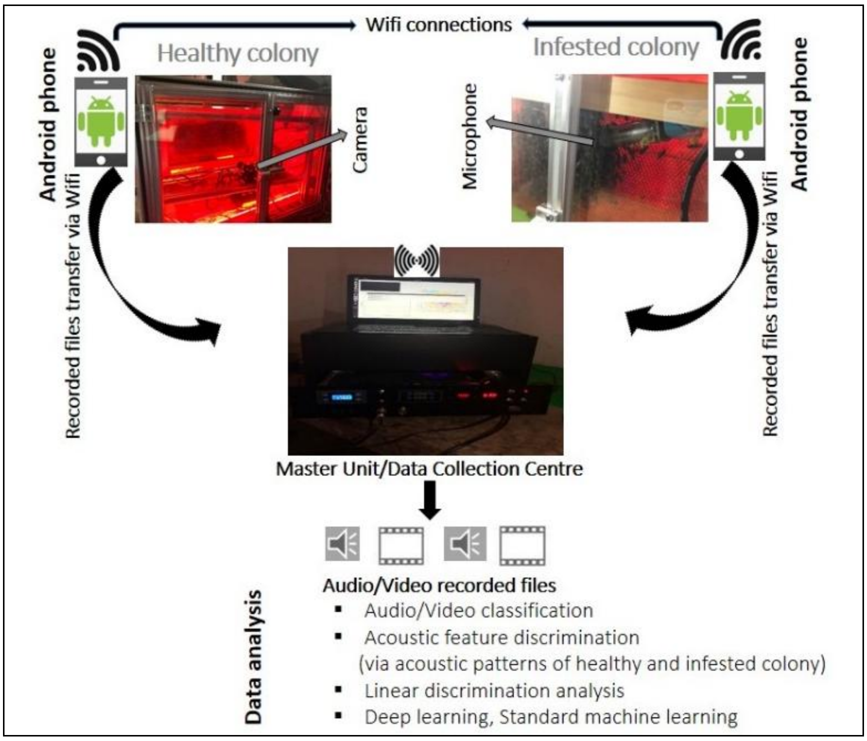

Fig 3: A setup to monitor beehive for pest infestations via acoustic recordings.

Fig 4: Pictorial view representing functionalities of CinixSoft Scheduled Voice Recorder for audio recordings and transmission. All windows (A-D) of this recorder exhibiting particular functions to perform: A0= tap this button to start recording, A1= as the recording starts, recording time will appear on screen, B2-5= these options can be used to edit event activation including "start and end time of recording, audio formatting (.wav, .3gp, and .mp4), to write purpose of recording on each recorded file, setting up particular number of weeks, and days for recording, 6C= long press of any recorded file results to appear "Share" option. D7-9= pressing share option will show all available options to transfer recorded files between connecting devices.

As android phones will be recording healthy and infested hives, they will automatically save and send audio recordings to the Master Unit or Data Collection Centre (Fig. 3). Thanks to this useful application, as it can automatically upload audio recordings to FTP server, email address as well as drop box without any human interaction (Fig. 4). The role of WiFi Direct (a new technology, Fig. 4D) which can assist us transfer files wirelessly is also highly significant. This technology transfer files like Bluetooth, but in blazing fast speed. This proposed system can prove as a useful tool to upgrade previously developed in-hive monitoring system. Audio recorded files transferred via proposed system can be analyzed to determine unique acoustic patterns or signatures which will predict the health status/ or infestation level of a colony (i.e. high, moderate, low, or none).

The recorded sound can be labelled, filtered and converted to a frequency spectrum by using certain software like Adobe Audition CS6, Avisoft SAS Lab Lite and Raven Pro version 1.5 (Bioacoustics Research program, Cornel Laboratory of Ornithology, Ithaca, NY, USA). Such software determine frequency as well as amplitude of the signal. Later, the data obtained from frequency and amplitude can be subjected to statistical discrimination. For the purpose of generating suitable classification function, filtered and summarized data can be statistically investigated via Standard canonical discrimination function plot (for more detail see [29]). Moreover, audio classification of these acoustic patterns can be accomplished by employing certain methods like Deep Learning and Standard Machine Learning. These methods employ Convolutional neural networks (CNNs), and Logistic regression, K-nearest neighbors, Support vector machine (SVM), and Random forest respectively [154, 32]. Apart from aforementioned methodologies, linear discrimination analysis can also be used for the purpose of audio classification [32]. These methodologies will permit a user to not merely detect a biological stressor (varroa mite's infection, or presence of hive beetles) but also quantify the level of infection within bee colony.

10. Conclusion and Recommendations

The declining populations of honeybee colonies, both acute and chronic, and the deleterious effects they have on the prognosis of agricultural crop production, prioritize them in current scientific attention. Research identifies insect invaders (insect pests, parasitoids, and predators) as dynamic threats that instigate non-lethal impacts on in-hive honey bees that disturb their social behaviors. Non-lethal impacts caused by SHBs include trophallactic solicitation, damage to cell caps of honeybee comb, and scavenger behavior against destabilized colonies, and such impacts caused by moths include web formation, galleriasis, nuisance, and mimicking piping sound of queen from *A. atropes*. Moreover, nonlethal impacts instigated by ants are: absconding of colony, nesting in dry hives resulting trouble for colony inspection. Furthermore, bee lice, for example, *B. coeca* larvae feed upon wax, pollen, and honey while adults disrupt bee's food supply to honeybee brood.

In social system, honeybees at colony level perform some defensive strategies like swarming, absconding, grooming (autogrooming and allogrooming), cooperative behavior (social immunity), spatial segregation, and social fever which help them fight against pest species. Honeybee hive defense behaviors against insect invaders (especially wasps and hornets) and pathogens are often intrinsically acoustic (bee-carpet, heat balling, piping, hissing or shimmering, and swarming) and therefore have characteristic frequency signatures that could be used for identification. However, such characterization has not yet been defined for other types of invertebrate invaders: Pests, predators and parasitoids of insect class *Insecta*. Preliminary research has begun into the effect of insect pests and ectoparasitic mite infection, and future inquiry could examine whether these frequency signatures vary with severity of infection, i.e. at non-lethal, threshold, and lethal levels. Better characterized are the acoustics of a beehive during confrontations with insect predators. For example, *A. mellifera* attacked by *V. orientalis* will produce a high 6 KHz frequency [30], but many questions still arise. First, does the predator also produce sound before or during the fight against the bees? In what frequency? Second, does the strength of the colony effect which acoustic frequencies are produced? Or the number of attacking organisms? With all of these considerations, we hope that recording of acoustic emissions during insect invaders, mite infection, and predator attack can offer worthwhile information to researchers, and serve as a reliable indicator by which beekeepers may assess apiary health. We also hope that our proposed idea of using android phones (installed with CinixSoft Recorder [Fig. 3&4]) will prove a useful diagnostic tool for researchers to collect acoustic data from colonies. This idea for sound collection can also be used to develop any novel in-hive monitoring system to identify infections severity, presence of hive beetles and insect predators via acoustic signatures. Considering the employment of developed monitoring system, our proposed monitoring system, and keeping in mind the role of apiary acoustics, we propose that the systems herein described could serve as a diagnostic test of whether insect pests, parasitoids and predators can degrade the social behavior of honeybees, and may become highly valuable as a key tool in assessing CCD.

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