Influence and Management of Colony Collapse Disorder (CCD) Damaging European Honeybee Apis mellifera

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ABSTRACT

The present investigation on the study of colony collapse disorder revealed the presence of *Nosema apis* and Foulbrood in honey bee colonies as a prominent factor responsible for the colony collapse in North Indian climatic condition, nevertheless other infection causing pathogens like Varroa mite, Red ant, application of the hazardous pesticide, wax eating moth were nil in the infection carrying colony. Humanly contrived supplements such as Vitamin B Complex 15 ml, and 75 mg Oxytetracycline three times a day each in five days interval was recorded to give the phenomenal improvement in honeybee efficiency. The minimal transformation after the first medication in all the parameter were beholden after 5 days of observation, but after 2nd dose of medicine usage a significant improvement was seen in flight activity, pollen foraging efficiency, the death rate was inspected to lower upto 2-8 bees per day after 10th day of 3rd dose of medication and observation their flight activity ranges from 33-62 per 10 minute, pollen foraging efficiency was 21-56 per minute, death rate 0-2 per day and worker and queen were almost normal in health.

Key Words: Honey bee, CCD, European Honey Bee, Disease, Disorder, Management

Introduction

One of the best means of survival to the people of rural area and tribal farmers is the art of beekeeping which has got an edge in recent years owing to its health benefits. The art of bee culture is getting more popular day by day in rural areas where abundance of nectar and flower are found. At the beginning of 21st century it was remarked that the bee pollination witnessed about $ 15 billion in added crop value (Oldroyd 2007). In major parts of Indian sub-continent throughout the year more than four native species (*Apis mellifera, Apis dorsata, Apis cerana, Apis florae and Melipona irridipennis*) of honey bee are found (Muttoo, 1956; Khanra and Mukherjee, 2018; Dar et al., 2020 a). The service rendered by bees is not only for yielding honey and waxes but they play a significant role in crop production enhancement as a result of

their foraging activity on several food crops (Dicks et al., 2016; Kapil, 1970). Indigenous methodology are employed by rural people to harvest honey from these species which leads to lower yield and quality deterioration keeping in consideration the low yield potential and migratory nature the bee species. The downfall can also occur due to the cumbersome nature of the orthodox process employed. Chaudhary, 2014 opined that the European honey bee species Apis mellifera was first introduced in the country in the late sixties owing to its easy handling, good honey yielder, these species are becoming popular in the country (Dar et al., 2017). Due to hard and persistent efforts made by government of India for the popularization of beekeeping amongst rural and tribal people through multiple rural upliftment programme, at present number of authentic and registered beekeepers in India have gone upto 7629 in number having 1133748 bee colony till March 2018 (https://nbb.gov.in/pdf/registered_beekeepers), where it scores 6th ranking with 4-5 thousand metric tons honey production in 2018 (Statista 2019). The plinth of the modern beekeeping relies on established refined scientific technology which advocates and employ the concepts of transportable frame-hive, honey extractor and the smoker (Sain V and Nain, 2017; Dar et al., 2016). When the immature stages of the honey bees matures, they join the worker class and are primarily responsible for care taking of the brood, building of hive, clean, repair, ventilate and when needed – cool or heat the hive (Winston, 1991). The sudden loss of bee population create a huge problem for the beekeepers boxes in spite of having abundant supply of pollen in the surroundings leading to fewer nurse bees and unproductive bee colony. In the bee box queen stops laying eggs as a result bee population decreases, and worker bees stops movement which have been described as colony collapse disorder (Francis Ratnieks and Norman L. Carreck, 2010; Vanengelsdorp et al., 2008; James D. Ellis et al., 2010; Vanengelsdorp et al., 2017; Roy et al., 2016; Hatjina et al., 2010;). All over the world scientist working on bees have proposed different cause of this disorder spanning from environmental issue to viral, bacterial, fungal pathogen, mite attack, nutrient scarcity, environmental constraint, and hazardous chemical insecticide application in foraging crops (Conte et al., 2010; Henry et al., 2012; Neumann P. and Carreck, 2010; Higes et al., 2008 and 2009; Francis et al., 2013; Martín-Hernández et al., 2008; Diana L. CoxFoster et al., 2007; Dussutour et al., 2012; Conte et al., 2010; Dar et al., 2020 b). Reports have been documented in which 90 per cent of the colonies have been lost and this phenomenon is observed in USA, Europe and other destinations. A lot of research aimed to find the cause of the colony collapse disorder (CCD), which is still mostly unknown.

A long list of biological, chemical, and environmental stressors has been linked to CCD, including Varroa mites (de Miranda et al., 2010), Israel acute paralysis virus (Cox-Foster, 2007; Blanchard et al., 2008), Nosema ceranae (Higes et al., 2008), and imidacloprid exposure which acts as a systemic neonicotinoid insecticide studied by (Maini et al., 2010; Girolami et al., 2009). The commercial exercise of migratory beekeeping, which often uses hives replacement to long distances to pollination sites, and malnutrition associated with monocultural food sources, have also been blamed for causing CCD authored by (Spivak et al., 2011; Dar et al., 2020 c).

In the late sixties of 19th century (1869) the first evidence of this disorder was recorded and an anonymous author perceived the loss of bees which left behind hives with ample quantity of honey. Speculations were done all over the country that the colony mortality was because of lack of pollen, poisonous honey and a hot summer (Anonymous, 1869). The abrupt emergence of colony collapse disorder (CCD) in the United States

during 2006-2007 and other countries raised the concern thus dwindling the importance of perennial pollinator globally (Engelsdorp et al., 2007; 2008; Bacandritsos et al., 2010). According to some theories (Oldroyd, 2007) about the reasons for CCD are presented, amongst which are diseases and parasites, in- and out-hive chemicals, genetically modified crops, even narrow genetic base and cold brood. It is concluded that CCD has a multifactorial cause, and the bees are immunosuppressed by different factors (Dar et al., 2021). What is more, a short list of the primary hypotheses is given in (Ellis, 2010), including chemical environmental pollution. The authors of (Coleman, 1996) has used a metagenomic approach to study the microflora in CCD hives, and examined possible contribution of different pathogens in CCD malady. They found that two dicistroviruses, Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV) of bees present in almost all of the CCD-affected hives and in very few of the control hives. The IAPV virus is found to be strongly correlated with a CCD occurrence. Nevertheless, in none of the control hives a combination of pathogens is detected, in contrast to their presence in the majority of the affected hives.

The common conclusion in the literature is that there is no single causative factor of CCD. Similarly, (Engelsdorp, 2009) performed a descriptive epizootiological study, where many quantitative variables, including bee body mass and protein analyses, morphometric measures, parasite and pathogen analyses as well as pesticide and genetic analyses are done. The research personalities also revealed that badly hit colonies are more likely to neighbour such colonies and vice versa, it is plausible CCD to be either contagious condition or to result from exposure to a common risk factor.There were another events concerning honeybee losses in the far and near history at different places (Finley et al., 1996; Silver, 1907), but in the early 2007, there were beekeepers who experienced 80–100% losses. Thus the disorder happened taken as one of the complex disease of honey bee colony (Vanengelsdorp et al., 2017). The scientific evidences proves multiple factor responsible for the difficulty in bee colony so many ways prove the most efficacious cure for CCD. However, several scientists opined that there are reports that Vit. B complex is very useful for a colonies to be free from the Nosema attack (Anderson and Dietz, 1976; Glavinic et al., 2017) and USDA has also granted an approval for the use of Tylosin, Oxytetracycline and Erythromycin in honey bee colonies to control bacterial diseases (Huang et al., 2013; Jan Suszkiw, 2005; Levy and Marshall, 2013). For the efficient management of the said malady we have tried our way best to inculcate curative measures of this disease, that will prove cost-effectiveness, easy in application and strenuous management of the disease.

Materials And Methods

In the month of November 2018 from the India, twenty-six boxes each containing honey with ten frames in every box and one healthy egg laying queen were procured under Biotech Kisan Hub project Funded By DBT. To ensure proper development and growth of the bee colony the bee boxes were checked regularly, bee movement and availability of nutrition in the colonies were also monitored at the regular intervals. There is abrupt downfall in bee population in six boxes out of 26 boxes though, no bee mortality have been seen in the nearby area. Initially bees were feeded with table sugar solution in the ratio of 4:1 in the plain water and 1/4 tablespoon of sulphur dusted in beehives for the control, despite all this remedy colony could not rejuvenated properly in without ruthless manner. Rest of the bee colonies in 20 boxes were performing good without any artificial diet supplement of sugar and sulfur. Apart from sugar and sulfur solution,

Vitamin B complex (Polybion SF) having Pyridoxine hydrochloride IP 0.75 mg, Thiamine hydrochloride IP 2 mg, Riboflavin Sodium Phosphate IP 2.5 mg, Nicotinamide 15 mg, D-panthenol, IP 3 mg Cyanocobalamin IP 2mg each in 5 ml of suspension along with 500 mg dose of Oxytetracycline (Terramycin) mixed in 100 ml of the suspension were fed to the five diseased boxes. For the data comparison, one infected box were kept as control and data was recorded thrice in a five days interval. After feeding the bee colonies with the medicine following behavioral activities of worker bees were recorded between 9 to 12 a.m early in the morning in the treated colony as well as in untreated colony including (diseased and healthy box) as per technique developed by Sharma (2014) is being put forward as:

Data Recording Parameters

The efficiency of worker bees: The efficiency of the honey bees was measured in terms of flight activity as it was observed that after every 10 minutes number of worker bees leaving the hive entrance and pollen foraging efficiency was measured in terms of number of worker bees returning with pollen loads.

Death rate: It was calculated by the number of dead worker bees in front of the hive.

Effect on egg laying: In the all the 5 diseased treated boxes number of freshly laid eggs were counted and one controlled and 6 healthy boxes for the comparative study.

Visual observation of another parasitic insect on the bee colony: Regular checking of infected colonies was done using a magnifier to record the presence of another destructive parasitic insect-like Varroa mite, Wax eating moth, Red ant.

Comparison of honey bees from the diseased as well as Healthy boxes: The magnification lens at 50X was employed for studying morphology like body size, color, wings and deformation of body parts.

Effect on bee motility in the comb and availability of bee bread in the comb: Workers movement was observed in each frame of every colony visually and availability of beebread was also observed daily in each box.

The shape and texture of dead bees and larvae within the comb: Dead bees collected from the colony were observed using 50X magnifiers, and the colour of the larvae within the bee wall.

Microscopy of infected bee for diagnostics: To study the fungal causal agents, microscopy of macerated ventricular portion of honey bee was done to check the infection due to American foulbrood or European foulbrood Ropey. The test was conducted by gentle piercing of matchstick in dead larvae as suggested by diagnosis of American foulbrood disease of honey bee brood. (http://agriculture.vic.gov.au/agriculture)

Effect on honey yield: Honey was extracted from the boxes on weekly basis, therefore yield of honey from the affected boxes and frames were also taken into the consideration as the amount of honey produced (in Kg)/week from each box.

Results And Discussion

The efficiency of workers bee in disease affected and healthy boxes

The range of flight activity of honey bees in control varies from 5-8 per 10 minute, their pollen foraging activity was calculated as 0-3 per 10 minute, mortality in the diseased box was 6-13 per day; however, egg laying was also almost negligible during the course of study. The minimal change was noticed after the first medication in all these parameters after five days of observation, but after 2nd dose of treatment significant improvement was noticed in flight activity as 8-31 per 10 minute and pollen foraging capability got enhanced to 4-23 per 10 minute nevertheless, the death rate after 10 days was reported to be 3-9 per days. When 3rd dose of medicine was given the observation was taken on 15 days and it was noticed that worker and queen were almost healthy, their flight activity ranges 33-62 per 10 minute, pollen foraging efficiency was 21-56/minute and death rate also decreases as 0-2 per day (Table 1A). All these parameters were also noted in similar time period in all the healthy boxes taken for comparative study was too given in (Table 1B). In almost all the observations i.e. 5th day, 10th day, and 15th day the flight liveliness was also observed which proves out to be similar and ranging from 61-94 per minute, pollen foraging activity ranges 23-54 per 10 minute and death rate was 0-4 in each day. The fecundity of the queen bee was ultimately high as 452-556 per day. Figure-1 gives a diagrammatic representation of disease attack, disease cycle and pathogen spillover on European honey bees turned into Colony Collapse Disorder.

Visual observation of another parasitic insect on the bee colony: There were no symptoms or physical presence of parasitic insect pest and damage caused by them like Red/Black ant, Varroa mite; however, wax eating moths were seen in the affected colony

Comparison of bees from diseased and healthy boxes on the basis of morphology: The bulged abdomen having the yellow pale colour, their head and antennae were carrying whitish growth were observed before treatment of worker bees from the infected boxes The morphological characters of bees from healthy boxes were different in terms of elongated abdomen of a worker bee, and black coloured head. After the collection from healthy boxes the larvae examined were creamy white in colour however, in case of the contaminated boxes they were pale brown to yellowish in external appearance. The shape of the bees turn out to be normal one after 15 days of medication, and whitish growth present on the top of the head region dwindled. Queen started fresh egg laying when dead larvae were thrown out.

Causal agents detection technique: Maceration of ventricular portion of honey bee observed through the light microscope revealed the presence of typical Nosema disease spores inside the honey bee body. Ropey test was also conducted to test the bacterial infection was negative though, the colouration of the immature stages and position gave the confirmity for the presence of European foulbrood disease.

Effect on bee motility in the comb and availability of bee bread in the comb

The worker honey bee is too lazy to move from one frame to other frame. Before treatment application the laziness was observed prior in all the boxes but astonishingly bees were agile enough in case of infected box and were directly striking to the face of handler, movement of the queen within the frame were slow,

meager amount of beebread was available in the comb despite abundance of rapeseed-mustard pollen and nectar before the treatment where bees of the healthy colony were relatively docile in that period of time, as it was seen in healthy bee boxes feed was very less during scanty pollen and nectar in summer season. Nevertheless, their alikeness to attack on the face was reduced significantly after treatment and worker bees initiated the pollen aggregation for making nutrition for queen is of prime necessity for egg laying.

Morphology of dead bees and larvae: The bees collected from the opening of bee box were examined for their abdominal texture which was white colored, rotting, both the antennae of bees were covered by white mass, distribution of brood in the comb was limited, nascent bee was dead in comb well before coming out from the brood shell before treatment in the infected box**.** The killing percentage of bees were highly decreased after treatment application, and formation new brood starts after 15 days of treatment.

Table 1A: Monitoring parameter of honeybee during the period of treatment A, Infected Colony B, Healthy colony

Activity	**Control**			**The efficiency of worker bees after treatment**																
	DB			**DB1**			**DB2**			**DB3**			**DB3**			**DB4**				
Days after	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**		
Flight activity	7	9	4	5	8	35	3	1	0	2	19	27	3	10	23	5	19	64		
Pollen foraging efficiency	0	2	2	0	23	36	1	3	4	0	10	26	2	9	20	0	4	21		
Death rate	13	9	6	7	4	0	4	1	0	9	3	0	11	7	2	23	14	3		
Egg laying	0	0	0	2	23	69	2	1	6	0	16	69	13	26	74	4	4	84		

DB denote Disease box

Table 1B: Monitoring parameter of honeybee during the period of treatment A, Infected Colony B, Healthy colony

Activity	**The efficiency of worker bees in healthy boxes**																	
	HB1			**HB2**			**HB3**			**HB4**			**HB5**			**HB6**		
Days after	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Flight activity	61	70	67	58	55	65	70	83	74	90	82	92	81	54	62	76	72	92

Pollen foraging efficiency	27	31	4	22	22	39	31	3	0	30	42	38	20	31	50	21	24	61
Death rate	02	00	01	00	4	00	04	02	0	01	02	00	02	01	02	01	11	02
Egg laying	498	488	490	490	486	494	47	479	471	470	473	501	501	501	495	540	559	499

HB denote healthy box

Effect on honey yield: At weekly intervals honey was regularly extracted from the healthy boxes and almost 5- 7Kg of honey from each box in a week were collected however, in case of diseased boxes with infection it was difficult as they cannot be sufficed naturally and even required artificial sugar diet to survive before the treatment. Although, bees behave normally after the treatment application and started foraging naturally. Honey bees are a social insect where a clear division of labor is seen and are reared in a migratory pattern. The migration of honey bee colonies is very important in India due to unavailability of pollen round the year and nectar in one place which compelled beekeepers to move from one location to other for their survival and continuous honey flow. These beneficial insect face a threat from large number of other bee species to survive, cope a period of stress when there is a shortage of food during summer and in between the period when two crops were grown in according to dynamic season. The regular change in diurnal temperature make them more prone to the attack by fungal and bacterial pathogen which ultimately result in bee mortality, less egg laying, restrictions in foraging and flight activity and bee colony gets distorted which leads to huge loss in terms of bee colony and the additional products received (honey and wax etc.) to the beekeepers (Bansal et al., 2013; Smith, 1953; Dangi et al., 2015). In the present investigation the presence of Nosema apis and European Faul Brood bacterium was prominent cause of the Collapse of bee colony In North Indian Condition. Daily activity of bees in contaminated boxes like number of flights, foraging was reduced, bee mortality was on higher side in comparison to the Healthy boxes. After treatment application of 15 ml Polybion SF syrup and 75 mg Oxytetrachloride per colony i.e. 3 doses each it was observed that after the first medication there was invisible change in all these parameters after 5th days of record observation, but after 2nd dose of medication there was significant improvement in flight activity of 8-31 per 10 minute, pollen foraging efficiency also got improved 4- 23 per 10 minute, death rate was 3-9 bees per day after 10th day when 3rd dose of medicine applied and observation was taken on 15 days worker and queen were almost normal in health, their flight activity ranges 33-62 per 10 minute, the pollen foraging efficiency calculated was 21-56 per minute and death rate also goes down. Comparative study done for healthy boxes were also noted in similar time period in all these boxes too given in flight activity in almost all observation i.e. 5th day, 10th day, and 15th day was similar and ranging from 61-94 per minute, pollen foraging activity ranges 23-54 per 10 minute and death rate was 0-4 per day. The egg laying capacity of queen was noted to be 452-556 per day which reflects significant improvement in no. of flight, foraging, honey collection, and egg laying. The migration honeybees during this period faced enough

stress due to lack of enough feed and stagnating in the bee boxes and hence become weaker. The parasitic microorganism like Nosema spp (Shimanuki, 1980; Dar and Ahmad, 2013; Castle, 2013), Bacterial pathogen, and viral pathogen (Gilliam et al., 1990) Mite (Kralj et al., 2006; Mathialagan et al., 2017) invaded feeble colonies more vigrously and become more liable to infection causing propagules. The summer period temperature in India is very hot and it can go more than 49^0C in some places and bees remain confined to the bee boxes, where external diet source in the form of syrup is fed for the survival of worker bees but queen stop egg laying due to shortage of shortage of pollen leading to starvation which are in conformity with earlier studies (Roulston and Goodell, 2011; Eccleston, 2007). The average life span of honey bees is 120 days approximately during the period of intense heat in north Indian region spanning from March to June in summers. In monsoon (July-September) there is a shortage of pollen and dearth in nectar availability in northern region of the country due to lack of agricultural activity. Due to the indeterminate use of herbicides and weedicides in an agricultural field it has resulted in killing of pollen bearing grasses but in recent years it has been observed that there is wide variation in diurnal temperature from the month of September- October (Night being cooler and days are hot) and also there is a problem of high relative humidity which forced honey bees for becoming prone to fungal bacterial and viral infection (Dar et al., 2018). However, the change in diurnal temperature was also correlated positively by Abrol (1998) and Holt (2014). Insecticide application as reported earlier is not the sole problematic cause for Colony collapse disorder in at least in north Indian Condition, as mustard being the main crop grown during Rabi season and sunflower in Zaid season where farmers should stop themselves to go for spray of multiple insecticide (Henry et al., 2012; Chensheng et al., 2014). The crop hardly needs one spray to control the mustard aphid in rapeseed-mustard crops whereas sunflower being unsprayed. Mango being highly insecticide spray crop in the field of study but European bee (Apis mellifera) does not visit the mango trees for foraging activity. The present investigation and worldwide report about the CCD studies put forward that CCD is considered complex disease caused by Nosema, bacterial and viral infection where multiple factors (both biotic and abiotic) plays the key role as described earlier in (Paxton, 2010; Evans et al., 2011; Conte and Navajas, 2008; Dussutour and Simpson, 2012; Cox-Foster et al., 2007; Vanengelsdorp *et al*., 2008 and 2010; Lecocq et al., 2016). Amongst the nutrient supplements Vitamin B Complex is considered best diet for honey bee to ward off the infection caused due to Nosema (Haydak and Palmer, 1942; Glavinic et al., 2017) which gets spread from the pollen in natural means. (Ptaszynska et al., 2012; Nazzi et al., 2012) further studied that in the scarcity of pollen, bees immune system become weak and are easily infected by Nosema, weather factor like variation in diurnal temperature and high humidity favour bacterial infection to the bee larvae (Eccleston, 2007). The chemical treatment application of Oxytetracycline @ 75mg per bee colony at three consecutive times in was proven to provide sufficient strength to the queen and workers to become infection free for efficient foraging exercise. Nevertheless, similar finding was also observed by Allipi *et al*., (1999) and Pettis et al., (2005). Further manifold pathosphere is faced by honey bees and their capacity to withstand these menace entrust upon commensalism, nutritional status, the junk of toxic chemicals and genetic resistance.

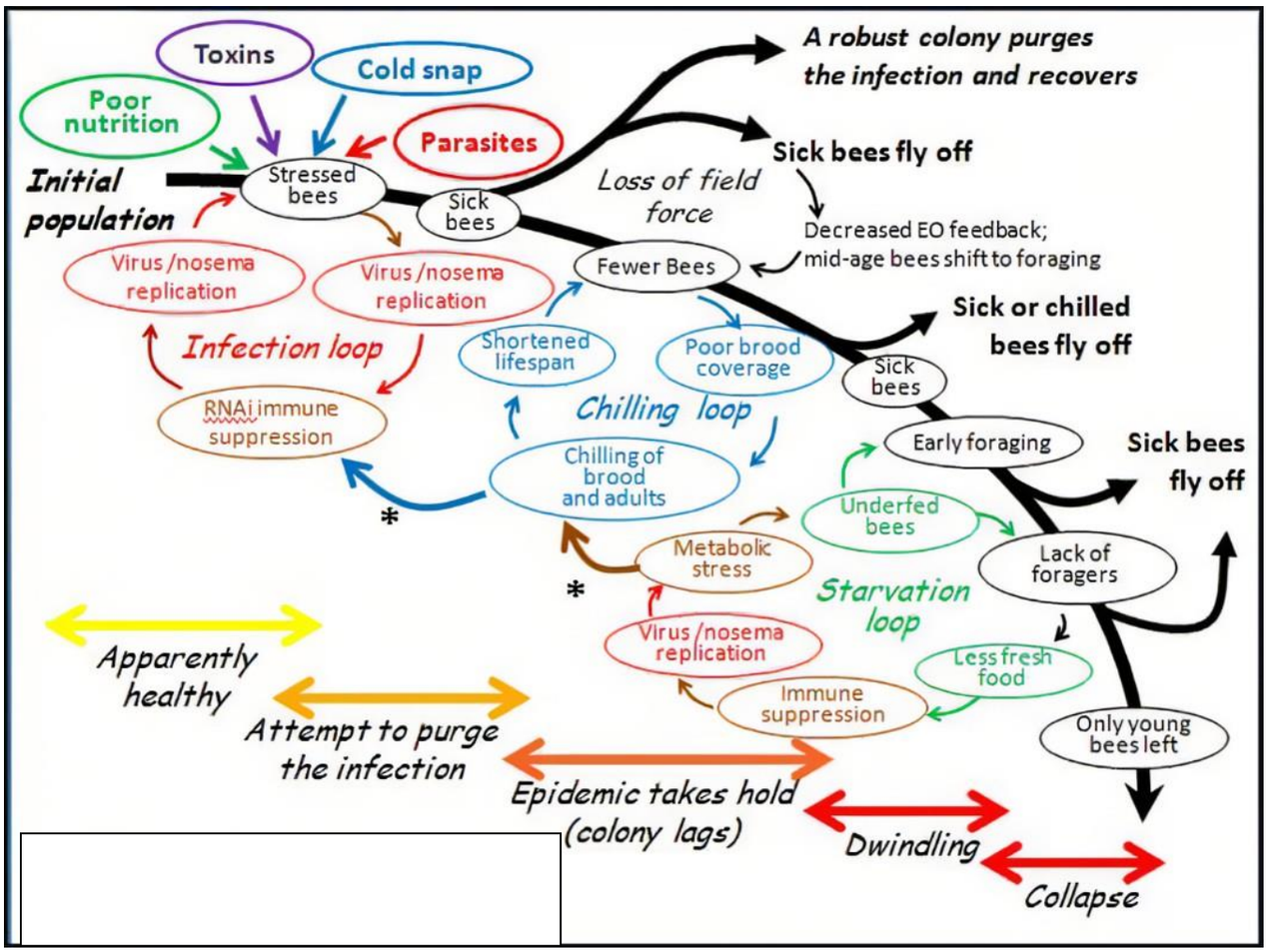

Figure -1: Diagrammatic representation of disease attack, disease cycle and pathogen spillover on European honey bees turned into Colony Collapse Disorder.

Note:

Unlike colony decline the CCD is caused by various issues such as queen health, varroa mite infestation, nutrition, and various diseases. In collapsed colonies, CCD is suspected when it is observed that few adult bees are physically present in the colony. Unlike with other acute causes of die-off such as pesticide exposure, few if any dead bees are found in or near the hive, as if the hive had simply been abandoned. A colony that has collapsed from CCD is generally characterized by all of these conditions occurring simultaneously: Presence of capped brood in abandoned colonies, Presence of food stores, both honey and bee pollen, Presence of the queen bee. If the queen is not present, the hive likely died because it was queenless, which is not considered CCD.

Precursor symptoms that may arise before the final colony collapse include: a) Inability to maintain current brood due to low workforce. b) Colony includes mostly young adult bees. c) Bees are reluctant to consume provided feed, such as sugar syrup and protein supplement

Conclusion:

The massive losses of honeybee colonies from the last 10-20 years have raised concern. Although there is still no consensus on what causes the phenomenon Colony Collapse Disorder, the model proposed in the paper helps to delineate the population dynamics of honeybee colonies. When diseased colonies were fed with Vitamin B Complex, and Oxytetracycline thrice each in five days interval there was significant improvement in honeybees efficiency. It accounts for both healthy and extinction behaviour of the honey bees, modeling CCD as a contagious infection brought to the hive by the foragers, caused by metabolic stress, environmental pollution and several other agents. It could support the beekeepers in managing the colonies in such a way to mitigate the severity or, hopefully, to completely eradicate the disorder's effect on the hives. A future work might include modification and improvement of the remedial measures to produce more precise description of the dynamics and a deeper analysis to suggest methodology for prevention of such dreadful diseases, impacting both environmental balance and economics of the bee keeper.

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