

Original Research Article

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Management of Colony Collapse Disorder in Honeybee (*Apis mellifera*): A Farmer's Friendly Approach

Vikash Chandra^{1*}, Arvind K. Singh¹, Sunil Singh¹, Ajay Kumar¹, Dheeraj K. Tiwari¹,
 Ratna Sahay¹, Ramesh C. Maurya¹ and Archana Singh¹

¹ICAR-Krishi Vigyan Kendra, Unnao-229881 Uttar Pradesh (India)

²Division of Agronomy, Banaras Hindu University Varanasi (India)

*Corresponding author

ABSTRACT

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The present study confirms the Presence of *Nosema apis* and European Foulbrood in Honey bee as a reason of the Collapsing Colony in north India condition, other pathogens like Varroa mite, Red ant, application of the hazardous pesticide, wax eating moth were not observed in the affected colony. These affected colony when fed with 15 ml Vitamin B Complex, and 75 mg Oxytetracycline thrice each in five days interval there was significant improvement in Honeybees efficiency After the first medication there was negligible change in all the parameter after 5th days of observation , but after 2nd dose of medication there was significant improvement in flight activity 11-34/10 minute, pollen foraging efficiency was also improved 5-24/10 minute, the death rate was reported to be 2-8/days after 10th days 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 31-64/10 minute, pollen foraging efficiency was 23-58/minute, death rate 0-2/day.

Introduction

Beekeeping accounts assets of viable income formation to the rural and tribal farmers and is getting popular in rural India where more than four native species (*Apis dorseta*, *Apis cerena*, *Apis florae* and *Melipona irridipennis*) of honey bee exist (Muttoo, 1956; Khanra and Mukherjee, 2018). Bees not only yield honey and waxes they also add in crop production too by their foraging activity on several food crops (Kapil, 1970; Dicks *et al.*, 2016). Traditionally rural people harvest

honey from these species using indigenous methodology leading to lower yield and deterioration in quality. Further being low yielder, migratory in nature it is cumbersome to rear these species. Another honey bee species *Apis mellifera* was introduced in the country in the late 1960s owing to ease in handling, and being honey yielder, these species are becoming popular in the country (Chaudhary, 2014). With continuous government efforts for the popularization of beekeeping in rural areas through different rural upliftment programme at present India

have 7629 registered beekeepers having 1133748 bee colony till March 2018 (https://nbb.gov.in/pdf/registered_beekeepers_16.08.2018.pdf), ranking 6th with 4 thousand metric tons honey production in 2018 (Statista 2019). Bee-keeping, now a day is established upon refined scientific technology employing the concepts of transportable frame-hive, honey extractor and the smoker (Sain V and Nain J 2017). However sometimes beekeepers face the problem of sudden loss of bee population from bee boxes in spite of having copious pollen in the surroundings leaving Queen, and few nurse bees leading to unproductive bee box, within the leftover bees in the boxing queen stop egg laying, therefore, bee population decreases, and worker stop movement. Which have been termed as colony collapse disorder (Vanengelsdorp *et al.*, 2017; Hatjina *et al.*, 2010; Roy *et al.*, 2016; Francis Ratnieks and Norman L. Carreck, 2010; Vanengelsdorp *et al.*, 2008; James D. Ellis *et al.*, 2010). Several group from all over the world have proposed different cause of this disorder ranging from environmental issue to viral, bacterial, fungal pathogen, mite attack, nutrient scarcity, environmental constraint, and hazardous 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; Dussutour *et al.*, 2012; Conte *et al.*, 2010; Martín-Hernández *et al.*, 2008; Diana L. Cox-Foster *et al.*, 2007). Therefore CCD is considered as a complex disease of honey bees (<http://npic.orst.edu/envir/ccd.html>, Vanengelsdorp *et al.*, 2017). The fact leads to the difficulty of the recommendation of a single remedy which can prove the most efficacious cure for CCD. However, literature study shows there are reports that Vitamin B complex is essential for a honey bee colony to be free from the Nosema attack (Anderson and Dietz, 1976; Glavinic *et al.*, 2017) and USDA has approved the use of Tylosin,

Oxytetracycline and Erythromycin in honey bees to control bacterial diseases (Jan Suszkiw, 2005; Levy and Marshall, 2013; Huang *et al.*, 2013). Keeping these researches in mind we have tried to develop cure of this disease, which is cost-effective, easy in application and effective in controlling the disease.

Materials and Methods

Twenty-six box honey bee boxes (having 10 frames of honey bees with one egg-laying queen each) were procured by Krishi Vigyan Kendra Unnao under Biotech Kisan Hub project Funded By DBT New Delhi in the month of November 2018, All the boxes were checked regularly for growth and bee population bee movement and availability of feeds in the colonies. Out of 26 in six boxes bee population started sudden declining further no bee mortality have been seen in the nearby area. To manage the problem initially we fed bee colony with table sugar solution (4:1) in water and 1/4 tablespoon of sulphur dusted in beehives, however colony could not rejuvenated in spite of having ample amount of rapeseed-mustard pollen within the reach of bees and other colonies in rest 20 boxes were performing well without any artificial sugar and sulfur supplement. Further based upon literature study we fed collapsing colony Vitamin B complex (Polybion SF manufactured by Merck) having Thiamine hydrochloride IP 2 mg, Riboflavin Sodium Phosphate IP 2.5 mg, Pyridoxine hydrochloride IP 0.75 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 this suspension were fed to the five affected boxes, one affected box were kept as control for data comparison, thrice in five days interval and data were recorded Before feeding and after feeding the medicine in the bee colony following

behavioral activities of honeybees (workers) were recorded between 9 a.m. to 12 a.m in the treated colony as well as controlled colony including (diseased and healthy box) as per methodology taken by Sharma N S. (2014) which is given as follows.

The parameter of data recording

The efficiency of worker bees: This was measured in terms of

Flight activity as the number of worker bees leaving the hive entrance per 10 min.

Pollen foraging efficiency measured as a number of worker bees returning with pollen loads per 10 min.

Death rate: Measured by the number of dead worker bees in front of the hive.

Effect on egg Laying No of freshly laid egg were counted in the all the 5 diseased treated boxes and one controlled one and 6 healthy boxes for the comparative study.

Visual observation of another parasitic insect on the bee colony: Infected colony were checked visually and using a magnifier to note the presence of another parasitic insect-like *Varroa* mite, Red ant, Wax eating moth

Morphological comparison of bees from both the diseased and Healthy boxes: Morphology like body size, color, malformation was studied using magnifier at 50X

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 noted in each box.

The texture of dead bees and larvae within the comb

Dead bees collected from the colony were observed using 50 X magnifiers, and larvae color within the bee well was also recorded.

Microscopy of infected bee and dead larvae and other diagnostic to find out causal agent

For finding the fungal causal agent microscopy of macerated ventricular portion of honey bee was done as the method described in Nosemosis of Honeybees, to check the infection due to American foulbrood or European foulbrood Ropey test was done 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/pests-diseases-and-weeds/animal-diseases/bees/diagnosis-of-americal-foulbrood-disease-of-honey-bee-brood>.

Effect on honey yield

Honey was extracted every week from the boxes, therefore honey yield from the affected and diseased boxes was also taken into the study 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

Flight activity of bees in control colony was ranging from 5-8/10 minute, their pollen foraging activity was 0-3/10 minute, mortality in the diseased box was 7-12/day, egg laying was also almost negligible during the period of study. After the first medication there was negligible change in all these parameters after 5th days of observation, but after 2nd dose of medication there was significant improvement

in flight activity 11-34/10 minute, pollen foraging efficiency was also improved 5-24/10 minute, the death rate was reported to be 2-8/days after 10th days 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 31-64/10 minute, pollen foraging efficiency was 23-58/minute, death rate 0-2/day (Table 1A). Whereas in the healthy boxes taken for comparative study was all these parameters were also noted in similar time period in all these boxes too given in (Table 1B) flight activity in almost all observation i.e. 5th day, 10th day, and 15th day was similar and ranging from 63-92/minute, pollen foraging activity ranges 20-52/10 minute and death rate was 0-5/day Queen egg laying capacity was noted to be 450-553/day.

Visual observation of another parasitic insect on the bee colony

No symptom/ physical presence of parasitic insect pest and damage caused by them like Red/Black ant, *Varroa* mite, and Wax eating moth were seen in the affected colony Figure 1 a-f.

Morphological comparison of bees from both the diseased and Healthy boxes

Before treatment worker bees from the infected boxes have swollen abdomen having the color of pale yellow, their head and antennae were carrying whitish growth Figure 2 a and b, in comparison to the elongated abdomen of a worker bee in the healthy boxes, and black head Figure 2 c and d. Larvae of the healthy boxes were milky white in color whereas in case of the infected box it was pale brown to yellowish in color. After 15 days of medication, bees turn to the normal shape and whitish growth in their head region disappeared. Dead larvae were removed and queen started fresh egg laying.

Microscopy and other diagnostic technique for the detection of causal agents

Light microscopy macerated ventricular portion of honey bee showed presence of typical *Nosema* spores in bee body. Further to test bacterial infection Ropy test was negative however larval color and position confirmed the presence of European foulbrood disease.

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

Sluggishness in worker to move from one frame to other was seen before treatment in all the boxes but surprisingly there were Agility of honey bee also noted in case of infected box and were directly attacking to the face of handler, queens movement within the frame were slow, there was scanty beebread available in the comb in the period of abundant rapeseed-mustard pollen and nectar availability before the treatment where workers of the healthy colony were relatively docile in that period of time, as it was seen in healthy boxes deprived of feed which during scanty pollen and nectar in summer period. However, after treatment their agility to attack was in the face was reduced significantly and workers started collecting pollen for making beebread's as feed for queen necessary for egg laying.

The texture of dead bees and larvae within the comb

The abdominal texture of dead bee collected from the opening of bee box was white colored, rotting, Antennae were covered by white mass, distribution of brood in the comb was scanty, nascent bee was dead in comb well before coming out from the brood shell before treatment in the infected box Figure 3a-f. After treatment, mortality was reduced, and fresh brood started developing after 15 days of treatment.

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

Table.1A

Activity	Control			The efficiency of worker bees after treatment															
	DB			DB1			DB2			DB3			DB4			DB 5			
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	
Days after 																			
Flight activity	6	8	5	7	11	41	8	34	64	2	25	31	5	13	26	7	23	64	
Pollen foraging efficiency	0	3	3	0	24	38	0	12	52	0	12	28	2	10	21	0	5	23	
Death rate	12	10	7	8	5	0	5	2	0	11	4	0	13	8	3	21	11	2	
Egg laying	0	0	0	2	25	71	7	23	75	0	18	68	11	23	72	3	25	81	

DB denote Disease box

Table.1B

Activity	The efficiency of worker bees in healthy boxes																	
	HB 1			HB2			HB3			HB4			HB5			HB 6		
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Days after 																		
Flight activity	63	72	68	57	56	64	69	82	75	92	89	94	85	83	96	77	73	94
Pollen foraging efficiency	26	32	43	24	24	38	30	32	52	32	42	38	22	30	51	20	25	63
Death rate	02	00	01	00	5	00	03	02	0	01	04	00	03	02	03	01	01	02
Egg laying	50	488	49	48	486	491	450	48	47	472	47	50	50	50	501	546	55	50
	1		2	9				2	2		3	3	5	3			3	1

HB denote healthy box

Fig.1 a and b Healthy bee colony c and d infected bee colony e dead bee in front of bee box opening, f, Infected live worker Bee



Fig.2 a and b Abdomen and Head of Infected c and d abdomen and head of a healthy honey bee

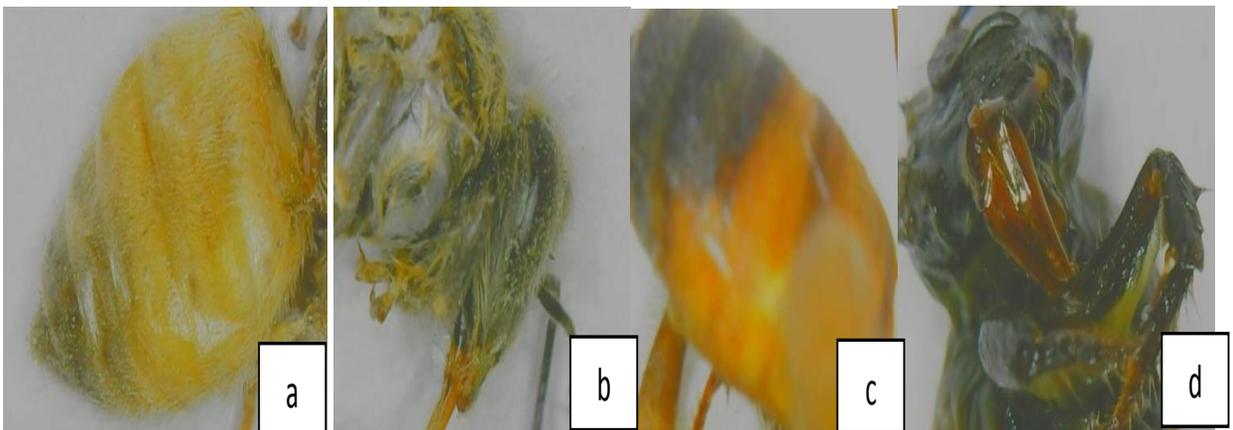
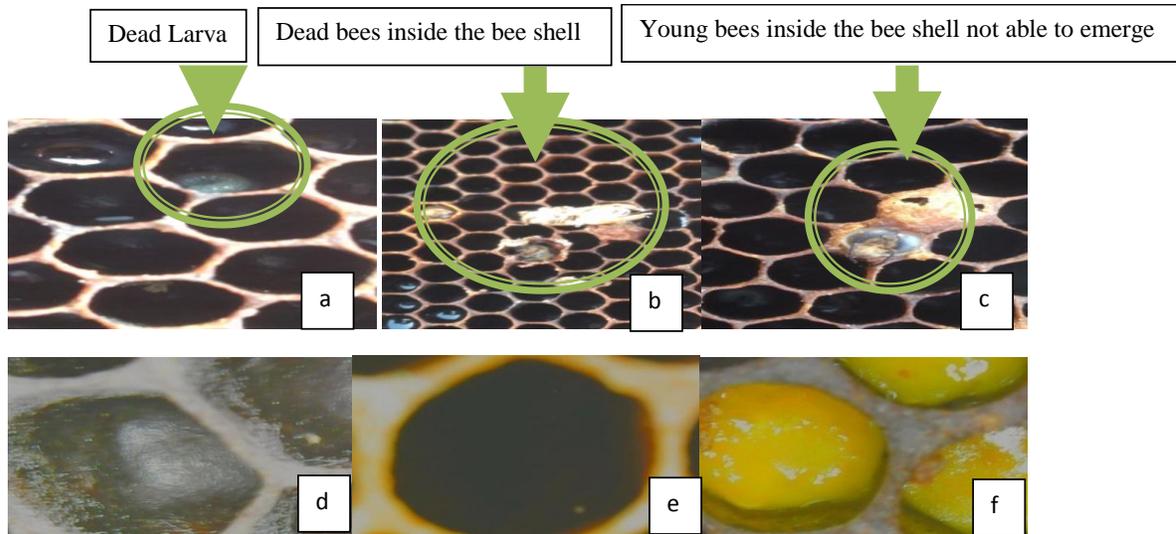


Fig.3 a. Dead Larva, b. Dead bees inside the bee shell, c. Young dead bees inside the bee shell not able to emerge, d. empty Bee Shell in the infected colony, e. Empty bee shell in healthy Colony, f. Beebread in healthy colony



Effect on honey yield

Honey was regularly extracted from the healthy boxes in the weekly interval, nearly 5-7Kg/ honey from each box per week were collected, whereas in case of infected boxes it was not possible as they even required artificial sugar feeding to survive before the treatment. However, after the treatment bees behave normally and started natural foraging and collection of honey.

Honey bees are a social insect with a clear division of labor, are reared in a migratory pattern in India due to unavailability of round the year pollen and nectar in one place and beekeepers are compelled to move from one location to other for survival of bees. These beneficial insect encounter large no of natural enemies to survive, cope a period of stress of unavailability of food during summer and in between the period of two crops grown in changing season and changing diurnal temperature which make them more prone to be attack by fungal and bacterial pathogen, causing bee mortality, stop egg laying,

foraging and flight activity are hampered and bee colony are collapsed leading to loss in terms of bee colony and bee byproduct yield (honey and wax etc.) to the beekeepers (Smith, 1953; Bansal *et al.*, 2013; Dangi *et al.*, 2015). The present study reveals the presence of *Nosema apis* and European Faul Brood bacterium as the cause of the Collapse of Bee colony In North Indian Condition. In the disease affected box daily activity of bees like Number of flight, Foraging was reduced, bee mortality was Higher in comparison to the Healthy boxes, after treatment *i.e.* 3 doses each consisting of 15 ml Polybion SF syrup and 75 mg Oxytetrachloride per colony it was observed After the first medication there was negligible change in all these parameters after 5th days of observation, but after 2nd dose of medication there was significant improvement in flight activity 11-34/10 minute, pollen foraging efficiency was also improved 5-24/10 minute, death rate was reported to be 2-8/days after 10th days 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

31-64/10 minute, pollen foraging efficiency was 23-58/minute, death rate 0-2%. Whereas in the healthy boxes taken for comparative study was all these parameters 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 63-92/minute, pollen foraging activity ranges 20-52/10 minute and death rate was 0-5/day Queen egg laying capacity was noted to be 450-553/day this reflect there was significant improvement in no of Flight, Foraging, Honey collection, and egg laying. During this migration honeybees face a period of stress due to lack of enough feed and stagnating in the bee boxes and thereby become weaker. These weak honey bee workers are more prone to be attacked by 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). In India during summer period temperature goes more than 49^oC in some places and bees remain confined to the bee boxes, where artificial sugar is fed for the survival of worker bees but queen remain starved due to lack of pollen and stop egg laying which supports earlier studies (Eccleston, 2007; Roulston and Goodell, 2011). The average lifespan of honey bees is approximately 120 days which is the period of summer intense heat in north Indian region from (March to June). In rainy season (July-September) to there is dearth of pollen and nectar availability in northern region of the country due to lack of growing agricultural crops having pollen and nectar and Use of weedicide in agricultural field kill the pollen-bearing grasses, the right time being October to End of February in northern region for Honey gathering but in recent Years it has been seen 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 forcing honey bees for becoming prone to fungal bacterial and viral infection change in diurnal temperature was also correlated positively by Abrol (1998) and Holt (2014). Insecticide application as reported earlier (Chensheng *et al.*, 2014; Henry *et al.*, 2012) does not seems to be the problem of Colony collapse disorder in at least in north Indian Condition, as Mustard is the main crop grown during Rabi season and sunflower in Zaid season where farmers do not need to spray any insecticide or need hardly one spray to control the mustard aphid in rapeseed-mustard crops whereas sunflower being unsprayed. Another highly insecticide sprayed crop being mango in the site of study but *Apis mellifera* does not visit to the mango for foraging activity. Present study confirm the earlier report from the worldwide studies as CCD being a complex disease having *Nosema* and bacterial and viral infection where multiple factors (both biotic and abiotic) plays the significant role as described earlier in (Paxton, 2010; Vanengelsdorp *et al.*, 2008 and 2010; Lecocq *et al.*, 2016; Evans *et al.*, 2011; Conte and Navajas, 2008; Dussutour and Simpson, 2012; Cox-Foster *et al.*, 2007). Vitamin B Complex is essential for honey bee to fight the infection of *Nosema* (Haydak and Palmer, 1942; Glavinic *et al.*, 2017) which is obtained from the pollen in natural means, in scarce pollen availability bees immune system become weak (Ptaszyńska *et al.*, 2012; Nazzi *et al.*, 2012) 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). Application of Oxytetracycline @ 75mg per bee colony thrice every time in equal dose have given sufficient strength to the queen and workers to become infection free and started foraging activity Similar finding was also observed by Allipi *et al.*, (1999) and Pettis *et al.*, (2005).

Further Honeybees face a manifold pathosphere and their capacity to withstand these menace confide upon commensalism, nutritional status, the hunk of toxic compounds, and genetic resistance and tolerance.

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