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Seasonal incidence of brood diseases in hives of Asian honey bee (*Apis cerana* F.) correlates with colony and weather parameters

Sapna Devi, Kiran Rana, Neha Negi, Harish Kumar Sharma and Meena Thakur

Abstract
Seasonal incidence of brood diseases in Asian honey bees, *Apis cerana* F. in relation to weather parameters was carried out at *A. cerana* apiary maintained by the Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan during January, 2019 to December, 2020. Pooled data on European foulbrood disease incidence showed that the disease incidence array from 0.80 to 17.50 per cent. The incidence was maximum (17.50%) in the month of July when temperature, relative humidity and rainfall were high and minimum in the month of March (0.80%) when temperature and relative humidity were low. The disease prevalence was assuredly correlated with temperature, colony strength and rainfall. Incidence of Thai sacbrood disease varied from 0.10 to 6.90 per cent and was maximum during the month of May (6.90%) when the colony strength, brood area and temperature were high and relative humidity and rainfall were low. The disease incidence was minimum in the month of October (0.10%) when temperature and rainfall were low. No incidence of brood diseases and Thai sac brood disease was observed in the month of December in both the years of study.

Keywords: seasonal incidence, *Apis cerana*, European foulbrood, Thai sacbrood, brood diseases

1. Introduction
Honey bees (Hymenoptera: Apidae) are vital pollinators for wildlife plants and agriculture crops (Klein *et al.*, 2007) [14]. Both *Apis cerana* and *Apis mellifera* species are raised to produce high value bee-products. *A. cerana* has specific advantages over *Apis mellifera* for its stronger tolerance in extreme climates (cold/hot weather) and lower cost of supplemental food (Peng *et al*., 1987; Oldroyd and Wongsiri, 2006; Xu *et al.*, 2009) [18, 17, 29]. Honey bees are susceptible to variety of diseases, pests and environmental threats. While it is impossible to identify single factor which on its own can account for all colony losses in all regions of the world. Among these factors, certain honey bee diseases and parasites have shown to play a significant role in increasing honey bee colony mortality and colony losses (Genersch, 2010) [11]. Honey bee diseases like American foulbrood, European foulbrood, Chalk brood, Sacbrood, Thai sacbrood, Nosema, Stone brood, Acarine and Varroasis are worldwide known diseases. European foul brood disease and Thia sacbrood disease are important bacterial and viral diseases which are prevalent throughout the country in *A. cerana* colonies (Rao *et al*., 2011) [22]. Thai sac brood virus (TSBV) infects larvae of the honey bee *A. cerana* resulting in failure to pupate and death. TSBV specifically infects *A. cerana indica* and is not known to cause infection on *A. mellifera* (Aruna *et al.*, 2014) [2]. European foulbrood disease (EFB) was noticed for the first time in *A. cerana indica* in the southwestern part of India during 1970 and then disappeared after killing 25-30 per cent of colonies (Diwan *et al.*, 1971) [8]. It reappeared after a long period of three decades in the northwest part during 2002, it spread during spring and summer and affected honey bee larvae before the capped stage exhibited symptoms like death of brood of *A. cerana* at larval and prepupal stages (Rana *et al.*, 2004, Fathy *et al.*, 2012) [20,9].

2. Material and Method
The present investigation on impact of weather parameters on seasonal incidence of brood diseases in *A. cerana* F. colonies were conducted during January, 2019 to December, 2020 in *A. cerana* F. colonies maintained by the Department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, which is situated at

33.3°N latitude, 70.70°E longitude and 1256 m above mean sea level (amsl) average climatic conditions of Himachal Pradesh. The incidence of diseases with respect to brood area, pollen area and weather parameters was recorded at monthly basis. Five colonies of *A. cerana* were selected randomly to record data on the incidence of brood diseases and colony parameters. Colony parameters *viz.*, brood area and pollen area were measured with the help of a measuring grid having squares, each square measuring one square inch (6.45 cm^2) on both sides of the frames. The brood area and pollen area was converted into cm^2 by multiplying the number of squares with a factor of 6.45. Field diagnosis of European foulbrood and Thai sacbrood disease was carried out on the basis of standard symptoms. The data on 100 brood cells in each of selected colony were examined for recording the incidence of brood diseases and the incidence of diseases were correlated with weather (temperature, relative humidity and rainfall) and colony parameters. The per cent brood infection was calculated as follows:

% brood infection in the colony = No. of infected brood cells/ Total no. of brood cells observed ×100

The statistics on incidence of European foulbrood and Thai sac brood were transformed using the square root transformation as per the method described by Gomez and Gomez (1986) [12], subjected to analysis of variance (ANOVA). The completely randomized block design with least significant differences between treatments were calculated taking all the possible combinations of factors. The treatment effect were tested at 0.05 per cent level of significance. The correlation of incidence of brood diseases were compared with colony and weather parameters by pearson correlation method. The incidence of brood diseases was correlated with weather (temperature, relative humidity and rainfall) and colony (brood area and colony strength) parameters. The data on ambient temperature and, relative humidity and rainfall were collected from the Department of Environmental science, College of Forestry Dr Y.S. Parmar University of Horticulture and forestry, Nauni, Solan for the period of experimentation.

3. Result and Discussion

3.1 European foulbrood disease

The advertences were recorded on seasonal incidence of European foulbrood and Thai sacbrood diseases in *A. cerana* colonies under stationary conditions from January, 2019 to December, 2020. The characteristic symptoms of European foulbrood disease (Fig 1) was recorded as, coiled larval position and spotty brood patteren (Bailey, 1961; Abrol and Ball, 2006; Foresgren, 2010) [3, 1, 10].

3.1.1 Incidence of European foulbrood disease in *A. cerana* colonies during the years 2019 and 2020

The data on seasonal incidence of European foulbrood disease during the years 2019 and 2020 is presented in Tables 1and 2. During the year 2019, the incidence of European foulbrood disease was statistically maximum (19.80%) in the month of May, 2019 when average colony strength and brood area were 4.44 bee frames and 1137.78 cm^2 and temperature, relative humidity and rainfall were 22.61°C, 44.00 per cent and 21.30 mm, respectively followed by June (13.40%) which was statistically at par with July (11.80%). The European foulbrood incidence in August was 7.20% which was statistically at par with April (5.00%) and September (4.80%) (Table 1). In the year 2020, European foulbrood disease incidence was statistically maximum (23.20%) in the month of July when average colony strength and brood area were 4.24 bee frames and 727.56 cm^2 and temperature, relative humidity and rainfall were 25.05 °C, 81 per cent and 278.10 mm, respectively followed by June (15.20%) which was statistically at par with May (11.80%) (Table 2). The minimum incidence of European foulbrood disease during 2019 was observed in the month of March (0.60%) when temperature, relative humidity and rainfall were low i.e. 13.45 °C, 54.00 per cent and 54.60 mm, respectively. During the year, 2020 least incidence (1.00%) of European foulbrood disease was observed in the month of March which was statistically at par with February (1.20%), January (1.40%), November (1.60%) and October (3.20%). The European foulbrood disease incidence disappeared in December, 2019 and reappeared in the same colonies during January, 2020 with 1.40% brood infection which was statistically same with February (1.20%) and March (1.00%) after that incidence increased up to July, 2020 and then started decreasing and was absent in December during both the years.

The ammunition on correlation of European foulbrood with colony and weather parameters revealed positive correlation (non-significant) with temperature (r= 0.55), colony strength (r= 0.14), brood area (r= 0.10), rainfall (r= 0.18) and negative correlation with relative humidity (r= -0.01) during the year 2019. In 2020, *A. cerana* colonies indicated positive correlation (significant) with temperature (r= 0.63) and non–significant correlation with colony strength (r= 0.32), rainfall (r= 0.55), relative humidity (r= 0.31) and with brood area (r= 0.01).

Pooled data on incidence of European foulbrood disease (Table 3) during both the years showed that European foulbrood incidence was maximum in the month of July (17.50%) when average colony strength and brood area were 4.11 bee frames and 808.83 cm^2 and temperature, relative humidity and rainfall were 24.44 °C, 80.00 per cent and 248.10 mm, respectively which was statistically at par with 15.80 per cent in the month of May and June (14.30%) followed by August (6.50%) which was statistically at par with September (6.30%) and April (6.10%). As the disease increases from April to July, the colony strength and brood area decreases. When the disease incidence was maximum, the temperature and relative humidity were high and rainfall was maximum. The high incidence of European foulbrood did not show significant reduction in colony strength and brood area. European foulbrood disease is reported to appear usually in spring and first half of summer season but in recent times its occurrence has not shown a clear dependence on season (Russenova and Parvanov, 2005) [24]. Negi (2017) [16] has observed maximum incidence of European foulbrood disease in the month of July, 2016 (23.00%), when colony strength and healthy brood area were 4.00 bee frames and 904.67cm^2/colony, respectively in *A. cerana* colonies at Nauni, Solan which supports the present findings. In a study conducted by Verma (2005) [28] maximum infection of European foulbrood (21.24%) in *A. cerana* was observed in the month of May, 2004 at Nauni, Solan due to high temperature and humidity. Rao (2009) [23] has recorded maximum incidence of European foulbrood (25.33%) in *A. cerana* in the month of August, 2007 at Katrain, Kullu.

Pooled data further showed that minimum incidence (0.80%) of European foulbrood was observed in the month of March

when average colony strength and brood area were 4.48 frames and 1444.80 cm^2 and temperature, relative humidity and rainfall were low i.e. 13.83°C, 58.00 per cent and 113.20 mm, respectively which was statistically at par with February (1.10%), November (1.50%) and January (1.60%). No incidence of European foulbrood was observed in the month of December in *A. cerana* colonies. Verma (2005) [28] and Negi (2017) [16] reported minimum incidence of European foulbrood disease in the same apiary of *A. cerana* during March. The disease incidence disappeared during December which finds support from the findings of Rao (2009) [23] and Negi (2017) [16] who also reported *A. cerana* colonies to be free from this brood disease during winter months. This may be due to less number of available nurse bees resulted in production of less amount of glandular food which caused the diseased larvae to appear starved during winter months and was easily detected and removed by bees (Bailey, 1977) [5]. Diwan *et al*. (1971) [8], Rana *et al.* (2004) [20] and Verma (2005) [28] reported similar type of occurrence and severity of diseases in *A. cerana* colonies in India.

The facts on correlation of European foulbrood with colony and weather parameters in *A. cerana* showed that the incidence of European foulbrood was significantly positive with temperature (r= 0.61). The incidence of European foulbrood was also positively correlated with colon y strength (r= 0.24), brood area ((r= 0.07), rainfall (r= 0.40) and relative humidity (r= 0.12), though non- significant.

3.2 Thai sacbrood disease

Thai sacbrood disease occurrence was recorded in *A. cerana* colonies which were kept at nauni during January, 2019 to December, 2020. The colonies were found free from disease in December during both the years. The brood was also observed for various symptoms of Thai sacbrood virus i.e., scattered pattern of brood area, tongue-like projection, change in colour of the brood, and sac-like structure of brood (Devaneson and Jacob, 2001; Srinivasan *et al.,* 2014) [7, 26] (Fig 1).

3.2.1 Incidence of Thai sacbrood disease in *A. cerana* colonies during the years 2019 and 2020

The data on seasonal incidence of Thai sacbrood disease during the years 2019 and 2020 is presented in Table 4 and 5. The data on incidence of Thai sacbrood disease observed in *A. cerana* colonies during 2019 varied from 0.60 to 6.60 per cent. Maximum incidence was observed in the month of May, 2019 (6.60%) when average colony strength and brood area were 4.44 bee frames and 1137.78 cm^2 and temperature, relative humidity and rainfall were 22.61 °C, 44 per cent and 21.30 mm, respectively which was statistically at par with April (4.20%) followed by September (3.00%) which was statistically at par with July (2.60%), February (2.00%), August (1.80%), January (1.60%), June (1.20%) and March (0.80%). Whereas during the year 2020, maximum incidence (7.20%) of Thai sacbrood disease was observed in the month of May when temperature, rainfall and relative humidity were high i.e. 22.05°C, 53.00 mm and 74.80 per cent, respectively and average colony strength and healthy brood area were 3.72 bee frames and 936.54 cm^2 and was statistically at par with April (5.40%).

In 2019, minimum incidence (0.60%) of Thai sacbrood disease was recorded in the month of November when average colony strength and brood area were 3.88 bee frames and 906.87 cm^2 and temperature, relative humidity and rainfall were 15.50°C, 62.00 per cent and 32.20 mm, respectively which was statistically at par with March (0.80), June (1.20%), January (1.60%), August (1.80%), February (2.00%) and July (2.60%). During the year 2020, minimum incidence was recorded in the month of October (0.20%), when average bee strength and brood area was 3.09 bee frame and 801.09 $cm^2$and temperature, rainfall and relative humidity were 20.40°C, 0.00 mm and 65.00 per cent, respectively. It was statistically at par with March (0.40%), June (0.60%), November (0.80%) and August (1.00%). There was no incidence of Thai sacbrood virus in the month of December, 2019 when the temperature and rainfall was low. The disease reappeared in the month of January, 2020 and was statistically at par with February, become maximum in the month of May, 2020 when temperature and rainfall were low, started decreasing May onwards till November and disappeared in December, 2020. No incidence of disease was recorded in December during both the years.

The facts on correlation of Thai sacbrood disease with colony and weather parameters showed that correlation was positive, non-significant with temperature (r= 0.42), colony strength (r= 0.28), brood area (r= 0.36), rainfall (r= 0.05) and negative with relative humidity (r= -0.26) during the year, 2019. In 2020, Thai sacbrood in *A. cerana* colonies indicated positive correlation (non-significant) with temperature (r= 0.28), colony strength (r= 0.44), brood area (r= 0.36), rainfall (r= 0.08) and negative with relative humidity (r=- 0.39).

Data on pooled incidence of Thai sacbrood disease observed in *A. cerana* colonies revealed that Thai sacbrood disease incidence varied from 0.10 to 6.90 per cent during 2019 to 2020 (Table 6). Significantly maximum incidence was observed in the month of May (6.90%) when average colony strength and brood area were high (4.08 frames and 1037.16 cm^2) and temperature was also high (22.33 °C) whereas, relative humidity and rainfall were low (48.50 per cent and 48.05mm, respectively). Negi (2017) [16] reported maximum incidence of Thai sacbrood disease in *A. cerana* in May, 2017. She has reported less percentage of brood infection (0.27% to 1.27%) in *A. cerana* colonies. These investigations got support from the findings of Rana and Rana (2008) [21] who has reported the incidence of sacbrood disease during April to May and maximum incidence in May with 1.6 to 15.0 per cent brood infection and 20 to 60 per cent colony infection when colony strength and broqod area started increasing at faster rate. During present studies the brood area and bee strength were high in February, March, April and May. Sharma (2002) [25] reported the incidence of Thai sacbrood disease during August to October, 2001 and again during March and July, 2002 in *A. cerana*. The occurrence of disease during spring to summer have also been reported by different workers (Chandel *et al.,* 1999, Hornitzky and Anderson, 2003; Rana and Rana, 2015) [6,13,19]. Kshrisagar *et al*. (1982) [15] recorded the incidence of Thai sacbrood virus during winter also in addition to early summer.

Least incidence of Thai sacbrood was recorded in the month of October (0.10%) which was statistically at par with March (0.60%) and November (0.70%). No incidence of Thai sacbrood disease was observed in the month of December in both the years. The disease in the present study started appearing in the month of January. The occurrence of sacbrood disease in build- up period is due to the existence of greater proportions of susceptible young adults and larvae in colonies (Bailey, 1968) [4]. Verma and Joshi (1985) [27] reported minimum incidence of Thai sacbrood disease in *A.*

cerana colonies in the month of October, 1984 in hills of Uttar Pradesh. Negi (2017) [16] observed the minimum incidence of Thai sacbrood disease in the month of November, 2016 (0.23%) in *A. cerana* colonies in the same apiary when the temperature and humidity were low i.e.15.80°C and 40.50 per cent, respectively and no rainfall.

The pooled data on correlation of Thai sacbrood incidence with colony and weather parameters in *A. cerana* colonies indicated positive non-significant correlation with temperature (r= 0.35), colony strength (r= 0.41), brood area (r= 0.37), rainfall (r= 0.05) and negative correlation with relative humidity (r=- 0.33).

Table 1: Incidence of European foulbrood disease in *A. cerana* colonies during January, 2019 to December, 2019

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	1.80 (1.67)	2.36	145.77 (12.08)	8.85	59.00	73.00
February	1.00 (1.41)	5.02	1153.26 (33.97)	10.34	63.00	103.10
March	0.60 (1.26)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	5.00 (2.45)	5.24	1849.90 (43.02)	20.03	49.00	36.80
May	19.80 (4.56)	4.44	1137.78 (33.68)	22.61	44.00	21.30
June	13.40 (3.79)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	11.80 (3.58)	3.98	890.1 (29.81)	23.82	79.00	218.10
August	7.20 (2.86)	2.45	647.58 (25.23)	24.43	79.00	225.80
September	4.80 (2.41)	3.60	957.18 (30.89)	23.48	77.00	151.40
October	3.20 (2.05)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	1.40 (1.55)	3.88	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	2.60	675.96 (25.79)	10.49	58.00	33.20
C.D $_{0.05}$	(0.53)	0.36	(5.18)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for European foulbrood =

Temperature × EFB incidence = 0.55
Relative Humidity× EFB incidence = -0.01
Rainfall × EFB incidence = 0.18
Colony Strength × EFB incidence = 0.14
Brood Area× EFB incidence = 0.10 (*Significant at 5%)

Table 2: Incidence of European foulbrood disease in *A. cerana* colonies during January, 2020 to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	1.40 (1.55)	2.08	245.10 (15.02)	9.10	68.00	168.30
February	1.20 (1.48)	4.44	1179.06 (33.80)	12.15	57.00	38.50
March	1.00 (1.41)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	7.20 (2.86)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	11.80 (3.58)	3.72	936.54 (30.5)	22.05	53.00	74.80
June	15.20 (4.02)	3.55	839.79 (28.94)	24.10	69.00	58.70
July	23.20 (4.92)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	5.80 (2.61)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	7.80 (2.97)	2.76	957.18 (30.87)	24.10	77.00	6.00
October	3.20 (2.05)	3.09	801.09 (28.20)	20.40	65.00	0.00
November	1.60 (1.61)	3.6	968.79 (30.67)	14.60	68.00	37.70
December	0.00 (1.00)	2.00	509.55 (22.18)	12.20	62.00	23.80
C.D $_{0.05}$	(0.64)	0.43	(5.79)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for European foulbrood =

Temperature × EFB incidence = 0.63*
Relative Humidity× EFB incidence = 0.31
Rainfall × EFB incidence = 0.55
Colony Strength × EFB incidence = 0.32
Brood Area× EFB incidence = 0.01 (*Significant at 5%)

Table 3: Pooled incidence of European foulbrood disease in *A. cerana* colonies during January, 2019 to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	1.60 (1.61)	2.22	195.43 (14.02)	8.98	63.50	120.65
February	1.10 (1.45)	4.73	1166.16 (34.16)	11.25	60.00	70.80
March	0.80 (1.34)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	6.10 (2.66)	5.38	1815.03 (42.61)	19.59	50.00	42.25
May	15.80 (4.10)	4.08	1037.16 (32.22)	22.33	48.50	48.05
June	14.30 (3.91)	3.32	880.42 (29.69)	24.92	58.50	78.60

July	17.50 (4.30)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	6.50 (2.74)	3.15	655.965 (25.63)	24.69	82.50	187.20
September	6.30 (2.70)	3.18	957.18 (30.95)	23.79	77.00	78.70
October	3.20 (2.05)	4.05	839.79 (29.00)	19.44	65.00	2.80
November	1.50 (1.58)	3.74	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	2.30	592.75 (24.37)	11.35	60.00	28.50
C.D $_{0.05}$	(0.49)	0.52	(3.51)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for European foulbrood =

Temperature × EFB incidence = 0.61
Relative Humidity× EFB incidence = 0.12
Rainfall × EFB incidence = 0.40
Colony Strength × EFB incidence = 0.24
Brood Area× EFB incidence = 0.07 (*Significant at 5%)

Table 4: Incidence of Thai sacbrood disease in *A. cerana* colonies during January, 2019 to December, 2019

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	1.60 (1.61)	2.36	145.77 (12.08)	8.85	59.00	73.00
February	2.00 (1.73)	5.02	1153.26 (33.97)	10.34	63.00	103.10
March	0.80 (1.34)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	4.20 (2.28)	5.24	1849.90 (43.02)	20.03	49.00	36.80
May	6.60 (2.76)	4.44	1137.78 (33.68)	22.61	44.00	21.30
June	1.20 (1.48)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	2.60 (1.90)	3.98	890.1 (29.81)	23.82	79.00	218.10
August	1.80 (1.67)	2.45	647.58 (25.23)	24.43	79.00	225.80
September	3.00 (2.00)	3.60	957.18 (30.89)	23.48	77.00	151.40
October	0.00 (1.00)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	0.60 (1.26)	3.88	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	2.60	675.96 (25.79)	10.49	58.00	33.20
C.D $_{0.05}$	(0.67)	0.36	(5.18)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for Thai sacbrood =

Temperature × EFB incidence = 0.42
Relative Humidity× EFB incidence = -0.26
Rainfall × EFB incidence = 0.05
Colony Strength × EFB incidence = 0.28
Brood Area× EFB incidence = 0.36 (*Significant at 5%)

Table 5: Incidence of Thai sacbrood disease in *A. cerana* colonies during January, 2020 to December, 2020

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	1.80 (1.67)	2.08	245.10 (15.02)	9.10	68.00	168.30
February	2.20 (1.79)	4.44	1179.06 (33.80)	12.15	57.00	38.50
March	0.40 (1.18)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	5.40 (2.53)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	7.20 (2.86)	3.72	936.54 (30.5)	22.05	53.00	74.80
June	0.60 (1.26)	3.55	839.79 (28.94)	24.10	69.00	58.70
July	3.40 (2.10)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	1.00 (1.41)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	2.60 (1.90)	2.76	957.18 (30.87)	24.10	77.00	6.00
October	0.20 (1.10)	3.09	801.09 (28.20)	20.40	65.00	0.00
November	0.80 (1.34)	3.6	968.79 (30.67)	14.60	68.00	37.70
December	0.00 (1.00)	2.00	509.55 (22.18)	12.20	62.00	23.80
C.D $_{0.05}$	(0.42)	0.43	(5.79)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for Thai sacbrood =

Temperature × EFB incidence = 0.28
Relative Humidity× EFB incidence = -0.39
Rainfall × EFB incidence = 0.08
Colony Strength × EFB incidence = 0.44
Brood Area× EFB incidence = 0.36 (*Significant at 5%)

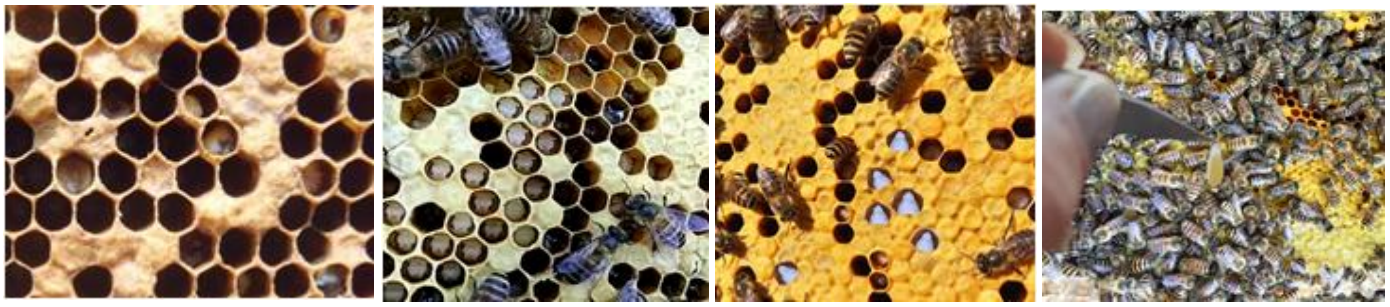
Table 6: Pooled incidence of Thai sacbrood disease in *A. cerana* colonies during January, 2019 to December, 2020

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Colony strength (bee frame)	Brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	1.70 (1.64)	2.22	195.43 (14.02)	8.98	63.50	120.65
February	2.10 (1.76)	4.73	1166.16 (34.16)	11.25	60.00	70.80
March	0.60 (1.26)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	4.80 (2.41)	5.38	1815.03 (42.61)	19.59	50.00	42.25
May	6.90 (2.81)	4.08	1037.16 (32.22)	22.33	48.50	48.05
June	0.90 (1.38)	3.32	880.42 (29.69)	24.92	58.50	78.60
July	3.00 (2.00)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	1.40 (1.55)	3.15	655.965 (25.63)	24.69	82.50	187.20
September	2.80 (1.95)	3.18	957.18 (30.95)	23.79	77.00	78.70
October	0.10 (1.05)	4.05	839.79 (29.00)	19.44	65.00	2.80
November	0.70 (1.30)	3.74	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	2.30	592.75(24.37)	11.35	60.00	28.50
C.D $_{0.05}$	(0.29)	0.52	(3.51)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) for Thai sacbrood =

Temperature × EFB incidence = 0.35
Relative Humidity× EFB incidence = -0.33
Rainfall × EFB incidence = 0.05
Colony Strength × EFB incidence = 0.41
Brood Area× EFB incidence = 0.37 (*Significant at 5%)



A. B. C. D.

Fig 1: Brood diseases of *Apis cerana* F. (a) European foulbrood infected larvae and sunken brood cappings (b) European foulbrood infected prepupae (c) Thai sacbrood infected larvae with tongue like projection (d) Thai sacbrood infected sac like larva

4. Conclusions

Incidence of brood diseases in *A. cerana* remained low to moderate without any significant effect on colony strength and its development. Incidence of diseases has correlation with temperature, colony strength, brood area, rainfall and relative humidity. European foulbrood disease is significantly correlated with temperature and non- significantly with colony strength, brood area, rainfall and relative humidity whereas thai sacbrood disease showed non- significant positive correlation with temperature, colony strength, brood area and rainfall.

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