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Efficacy of plant products on sacbrood virus attacking *Apis cerana indica* Fabricius

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Abstract
Honey bees are one of the most important beneficial insects having role in the crop improvement. It is affected by various calamities like pest and pathogens. The sacbrood virus (AcSBV) is a serious disease infecting *A. cerana indica* causing colony loss throughout the nation and south India in particular. Experiment was conducted on the efficacy of plant products showed that provision of 2 g of *Phyllanthus niruri* L. extract in 250ml sugar solution combined with modified shook swarm method recorded the lowest number of infected larvae per thousand brood cells after third round of treatment at 4 days interval and prevented absconding of colonies. This treatment was followed by treatments with extracts of *Ficus religiosa* L., *Carica papaya* L. and *Azadirachta indica* L. With the supplementary sugar feeding, there was an increase in the adult population in the honey bee colonies that recovered from the disease. Overall, it could be concluded that, honey bee colonies fed with 2g of *P. niruri in 250ml* sugar solution combined with modified shook swarm method was effective for both the recovery of disease and increase in brood rearing and adult population.

Keywords: Sacbrood virus (SBV), management, botanicals, *Phyllathus*, modified shook swarming

1. Introduction
Honey bees, economically important insect and considered to be good pollinator. They are usually affected by virus, fungi, protozoa and other insect pests. Among them, the sacbrood virus (AcSBV) is a serious problem in *A. cerana indica* which leads to heavy loss to bee keepers throughout India. It was highly virulent in north eastern and northern India, being responsible for heavy losses to beekeeping killing over 95 percent of *A. cerana* colonies [1- 3]. In Mahabaleshwar, this virus infected the brood of *A. cerana* and caused destruction of 21 colonies out of a total of 44 colonies in summer of 2008. Among all viral honey bee diseases, 17.6 percent of diseased bees were reported to be infected with SBV in Korea in 2009 [4].
In Kerala, the catastrophic outbreak of SBV disease resulted in the destruction of more than 90 percent of the then existing bee colonies causing a drastic drop in honey production during 1991-92 [5]. At present the disease infects 5 to 30% of the colonies in Tamil Nadu [6]. Several methods have been developed, such as electron microscopy [7] (Break *et al.*, 1963), Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [8, 9] and Reverse Transcriptase Loop Mediated Isothermal Amplification RT-LAMP [10, 11] for the diagnosis of the viruses. Very little work was done on the management of the SBV. Since then the management has been very challenging to the researchers as the causal organism is a virus and no curative measure works well to contain the disease during an outbreak. With this background, the present study was conducted on the management of sacbrood virus infecting honey bees using plant extracts.

2. Materials and methods
Aqueous extracts were prepared with different parts of plants namely *Phyllanthus niruri* L., *Azadirachta indica* L., *Ficus religiosa* L. *and Carica papaya* L. as mentioned in Table 1. The samples were crushed in a grinder for complete herbal extraction from the sample. Two grams of the plant part was added to 250 ml of water and the sample was boiled for 10 minutes to obtain aqueous extract. About 250 grams of sugar was added to it and allowed for further boiling. This final solution was fed to one colony by pouring in a glass bottle, covering with a two-layer muslin cloth, securing with rubber band and providing to honey bees in inverted manner inside the hive. Three rounds of such treatments were given at four days interval. Shook swarm method that is generally recommended for brood disease management involves shaking the bees to a fresh hive leaving behind all the combs of infected honey bee colony in

the old hive. In this study, we modified the shook swarm method in order to decrease the probability of deserting by honey bees which is one of the fallouts of the shook swarm method. In the modified shook swarm method, the brood comb infected by honey bees alone were removed (instead of removing all the combs) and in its place fresh brood combs from healthy hives provided. In T5 the modified shook swarm method was combined with *P. niruri* treatment which was based on the promise shown by these treatments in preliminary experiments (data not shown). Twenty four honey bee colonies showing typical virus symptoms were selected and four colonies per treatment were used as replication. Four more healthy colonies were selected and used as healthy control.

Table 1: Plant products used for the management of sacbrood virus of *A.cerana indica*

Treatment No.	Plant	Part of plant used	Dose (in grams /250 ml provided with sugar solution)
T1	*Phyllanthus niruri* Hook (Keezhanelli)	Matured whole plant	2.0
T2	*Azadirachta indica* A.Juss (Neem)	Bark	2.0
T3	*Ficus religiosa* L. (Peepal)	Roots	2.0
T4	*Carica papaya* L.(Papaya)	Leaves	2.0
T5	Modified shook swarm method with *P. niruri*	Matured whole plant	2.0
T6	Untreated diseased control	-	-
T7	Untreated healthy control	-	-

Data were recorded on the number of infected larvae per 1000 brood cells prior to treatment and four days after each treatment and the adult population per colony and total brood cells per 1000 cells were recorded on 15, 30 and 60 days after third treatment (DAIIIT). The adult bee population was arrived using the following formula.

Total number of bees in a colony = (No. of brood frames covered by bees × 1000) + (No.of super frames covered by bees x 750). The adult population was expressed in 1000s.

3. Results

3.1 Effect of antiviral plant extracts on AcSBV infected larvae per 1000 brood cells

The effect of different herbal extracts at 2 gm / 250 ml sugar solution provided thrice at 4 days interval was studied on virus infected honey bee colonies in terms of number of infected larvae per 1000 cells at 4 days interval. The adult bee population and brood development was studied in the surviving colonies on 15, 30 and 60 days after third treatment (DAIIIT).

It was observed that, the number of infected larvae per 1000 brood cells before imposing treatments ranged from 30.3 to 35.3 and was statistically on par. On the fourth day after third round of treatment, the number of infected larvae per thousand brood cells was lowest (18.5) in the colonies managed by modified shook swarm method and combined with provision of 2g of *P. niruri* extract in 250ml sugar solution. The least effective treatment was *A. indica* (58.1 larvae/1000 brood cells). In the untreated diseased check all the four colonies absconded due to the severity of the disease (Table 2). Similar trend was observed with respect to other days of observation namely 4 DA IT and 4 DAII T.

3.2 Effect of antiviral agents on adult bee population of Indian honey bees

The adult bee population on 60 DAIIIT was highest (8.8 thousand/ colony) in the colonies managed by modified shook swarm method combined with provision of *P. niruri* extract in sugar solution. The least effective treatment was *A. indica* (5.4 thousand/ colony). In the untreated diseased check, all the four colonies absconded due to the severity of the disease (Table 3). Similar trend was observed with respect to other days of observation namely 15 and 30 DAIII T.

3.3 Effect of herbal plants on brood rearing in Indian honey bees

The total number of brood cells (which is an indicator of colony growth) on 60 DAIIIT was highest (5197.50 per colony) in the colonies managed by modified shook swarm method combined with provision of *P. niruri* extract in sugar solution.

The least effective treatment was *A. indica* (2898.0 per colony). Other treatments namely *C. papaya* and *F. religiosa* at 2g / 250 ml were also effective. In the untreated diseased check all the four colonies absconded due to the severity of the disease (Table 4).

In the untreated healthy check, 4315.50 brood cells per colony was recorded. Similar trend was observed with respect to other days of observation namely 15 and 30 DAIII T.

4. Discussion

In order to study the antiviral effects of plant extracts on the infection caused by AcSBV, medicinally important plants possessing various phytochemicals that can act on viruses were used for the preparation of extracts and were tested on honey bee colonies infected with the virus.

It was observed that the honey bee colonies managed by modified shook swarm method combined with provision of 2 g of *P. niruri* extract in 250ml sugar solution recorded the lowest number of infected larvae per thousand brood cells on the fourth day after third round of treatment which was 50 to 60 percent lower than the diseased control. The least effective treatment was *A. indica* (Fig. 1). The adult population as well as the total brood cells on 60 DAIIIT which are indicators of colony growth was highest in the colonies managed by modified shook swarm method combined with provision of 2 g of *P. niruri* extract in sugar solution (Fig. 2). The least effective treatment was *A. indica*. In the untreated diseased check all the four colonies absconded due to the severity of the disease.

Earlier studies have indicated that *P. niruri* and *A. indica* were used for the management of AcSBV [12, 13] while *F. religiosa* and *A. indica* by different scientists [14]. They have reported effective control over the disease. The phytochemicals present in the herbal extracts can be the reason for the effectiveness against the virus. Many plants contain ribosome-inactivating proteins (RIPs) that alter ribosomal function in the infected cell and inhibit viral protein synthesis [15]. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, identified from various parts of *P. niruri* and its extracts have been proved to have therapeutic effects in many clinical studies [16]. Bark of peepal tree contains tannins, saponin, flavonoids, steroids, cardiac glycosides and terpenoids are biologically active

against virus particles [17-19]. Quercetin has been reported to be the most abundant and active flavonol [20]. Literature on the qualitative phytochemical analysis of papaya leaves revealed the presence of all the phytochemical including glycosides, alkaloids, saponins, flavonoids, proteins except steroids and tannins in the leaf of papaya [21-23].

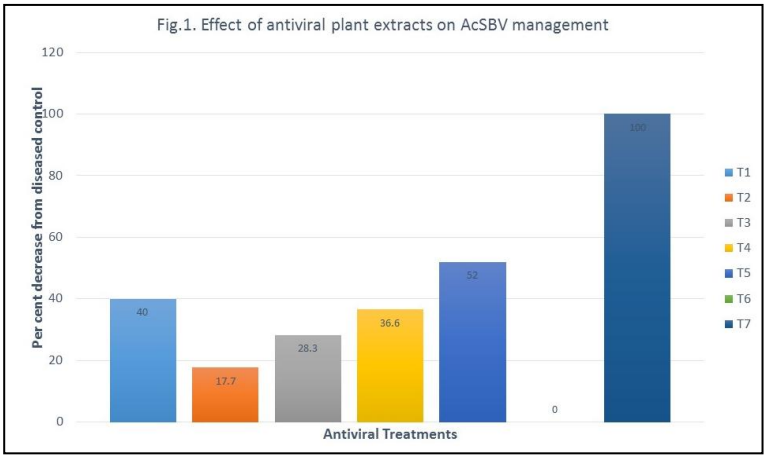
Table 2: Antiviral effect of plant extracts on AcSBV management - number of infected larvae /1000 brood cells

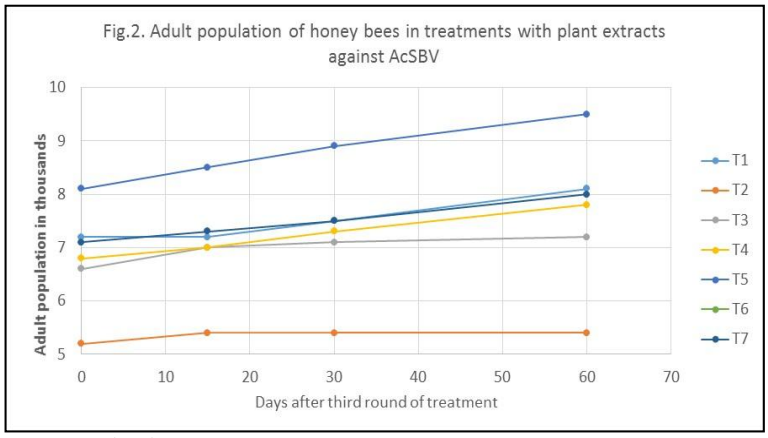
Treatment	Dose (g/250 ml)	Number of cells with infected larvae per 1000 brood cells*					Percent decrease from diseased control
		0 DAT	4 DA IT	4 DAII T	4 DAIII T	MEAN	
P. niruri	2.0	30.3 (5.64)	48.3 (6.98) b	62.8 (7.95) b	28.3 (5.36) b	42.4 (6.46) b	40.0
A. indica	2.0	32.3 (5.72)	60.5 (7.81) c	88.3 (9.42) e	51.5 (7.21) e	58.1 (7.62) e	17.7
C. papaya	2.0	35.3 (5.98)	58.8 (7.70) c	73.0 (8.57) d	35.5 (6.00) d	50.6 (7.06) d	28.3
F. religiosa	2.0	31.5 (5.65)	47.3 (6.91) b	68.8 (8.32) c	31.5 (5.66) c	44.8 (6.74) c	36.6
Modified Shook swarm method with *P. niruri*	2.0	35.3 (5.98)	38.3 (6.22) a	43.5 (6.63) a	18.5 (4.36) a	33.9 (5.80) a	52.0
Untreated diseased check	-	34.5 (5.92)	64.0 (8.03) d	92.0 (9.62) f	92.0** (9.62)	70.6 (8.30)	0.0
Untreated Healthy check	-	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	100.0
Mean	-	23.7 (4.92) A	37.7 (6.18) C	51.0 (7.18) D	30.6 (5.58) B		
CD (0.05) T CD (0.05) P CD (0.05) T x P		0.10 0.08 0.19					

No. of days after first round of treatment (DAT), days after second round (DAIIT), days after third round (DAIIIT); antiviral treatments given on 0,4 and 8 days

*Mean of four honeybee colonies. In a column, means followed by a common alphabet are not significantly different by LSD (p= 0.05); in a row, means followed by a common uppercase alphabet are not significantly different by LSD (p= 0.05)

**Colonies absconded





T1 *P. niruri*
T2 *A. indica*
T3 *C. papaya*
T4 *F. religiosa*
T5 Modified Shook swarm method with *P. niruri*
T6 Untreated diseased check
T7 Healthy check

Table 3: Antiviral effect of plant extracts on AcSBV management - adult bee population / colony

Treatment	Dose (g/250 ml)	Adult population ($X10^3$)				
		0 DAIIIT	15 DA III T	30 DA III T	60 DA III T	MEAN
P. niruri	2.0	7.2 (2.77) b	7.2 (2.79) bc	7.5 (2.82) b	8.1 (2.94) b	7.5 (2.83) b
A. indica	2.0	5.2 (2.40) e	5.4 (2.44) e	5.4 (2.43) d	5.4 (2.44) e	5.4 (2.42) e
C. papaya	2.0	6.6 (2.67) d	7.0 (2.74) d	7.1 (2.76) c	7.2 (2.78) d	7.0 (2.74) d
F. religiosa	2.0	6.8 (2.70) c	7.0 (2.75) cd	7.3 (2.80) b	7.8 (2.88) c	7.2 (2.78) c
Modified Shook swarm method with *P. niruri*	2.0	8.1 (2.94) a	8.5 (3.00) a	8.9 (3.07) a	9.5 (3.17) a	8.8 (3.04) a
Untreated diseased check**	-	0.0 (0.71) f	0.0 (0.71) f	0.0 (0.71) e	0.0 (0.71) f	0.0 (0.71) f
Untreated Healthy check	-	7.1 (2.75) b	7.3 (2.80) b	7.5 (2.82) b	8.0 (2.92) bc	7.5 (2.82) b
Mean	-	4.9 (2.31) D	5.1 (2.36) C	5.2 (2.39) B	5.5 (2.45) A	
CD (0.05) T CD (0.05) P CD (0.05) T x P		0.02 0.02 0.04				

No. of days after third round of treatment (DAIIIT); antiviral treatments given on 0,4 and 8 days

*Mean of four honeybee colonies. In a column, means followed by a common alphabet are not significantly different by LSD (p= 0.05); in a row, means followed by a common uppercase alphabet are not significantly different by LSD (p= 0.05)

**Colonies absconded

Table 4: Antiviral effect of plant extracts on AcSBV management - number of brood cells / colony

Treatment	Dose (g/250 ml)	Total number of brood cells				
		0 DAT	15 DA III T	30 DA III T	60 DA III T	MEAN
P. niruri	2.0	4032 (63.45) b	3906 (62.49) bc	4347 (65.92) b	5292 (72.75) b	4394.25 (66.15) b
A. indica	2.0	2646 (51.41) d	2709 (52.04) d	2961 (54.24) c	3276 (57.24) d	2898.00 (53.73) d
C. papaya	2.0	3591 (59.92) c	3654 (60.43) c	4095 (63.99) b	4347 (65.91) c	3921.75 (62.56) c
F. religiosa	2.0	3654 (60.43) c	3654 (60.43) c	4158 (64.47) b	4725 (68.67) bc	4047.75 (63.50) c
Modified Shook swarm method with *P. niruri*	2.0	4410 (66.39) a	4599 (67.81) a	5355 (73.17) a	6426 (80.16) a	5197.50 (71.88) a
Untreated check**	-	0.0 (0.71) e	0.0 (0.71) e	0.0 (0.71) d	0.0 (0.71) e	0.0 (0.71) e
Untreated healthy check	-	3717 (60.94) c	4158 (64.47) b	4158 (64.47) b	5229 (72.30) b	4315.5 (65.54) b
Mean	-	2625.0 (51.24) C	2700.0 (51.97) C	2985.0 (54.64) B	3487.5 (59.06) A	
CD (0.05) T CD (0.05) P CD (0.05) T x P		1.48 1.21 2.97				

No. of days after third round of treatment (DAIIIT); antiviral treatments given on 0,4 and 8 days

*Mean of four honeybee colonies. In a column, means followed by a common alphabet are not significantly different by LSD (p= 0.05); in a row, means followed by a common uppercase alphabet are not significantly different by LSD (p= 0.05)

**Colonies absconded

5. Conclusion

Even though, the plant products with antiviral principle products could not cure SBV infection in *A. cerana indica* fully, but they were found to keep the disease under check to a level of 50 percent. This helps the beekeepers not only in preventing the honey bees from absconding but involves in minimising the loss to them.

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