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Effect of screened bottom and sugar powder dusting against *Varroa jacobsoni* Oudemans management in Indian bee, *Apis cerana* F. (Hymenoptera: Apidae)

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Abstract
Honey bee colonies continue to decline in numbers all over the world due to various stressors, regular occurrence of the ectoparasitic mite *Varroa jacobsoni* Oudemans, causes concern in Indian honey bees, *Apis cerana* F. An experiment with *A. cerana* colonies was conducted in 2018 to study the effect of keeping an open screened bottom over the solid bottom board and of dusting powdered sugar as physical methods to manage *V. jocobsoni* in *A. cerana.* During the 5 month study period, the physical methods caused more *V. jacobsoni* adults to fall down and get trapped on the solid bottom board through the open screen than those in control hives. However, there was no significant difference in the number of nymphs and adults of *V. jacobsoni* found inside the brood cells of both drones and workers. Hence, the dusting of powdered sugar was not alone effective and it is effective when incorporated with integrated management practices. The effect of sugar powder dusting is discussed.

Keywords: *A. cerana*, screened bottom, sugar powder dusting, *V. jacobsoni*

Introduction
Wonderful eusocial insects, honeybees play a key role in crop pollination. While insects pollinate about 35 per cent of the human food-producing crops, honeybees alone provide pollination service in over 90 per cent of these crops (Klein *et al.*, 2006) [9]. However, managed honeybee colonies all over the world are on the decline due to stress from factors such as pesticide poisoning, pollutants, parasites, diseases and malnutrition (Klein *et al.*, 2017; Yasuda *et al.*, 2017) [10, 17], often phrased as Colony Collapse Disorder (CCD) (Mcmenamin and Genersch, 2015) [13]. Notable among them is the ectoparasitic mite, *Varroa jacobsoni* (Oudemans) (Mesostigmata: Varroidae) detrimentally associated with many physical and physiological effects at the individual bee and colony levels (Finley *et al.*, 1996) [5]. Repeated feeding by *Varroa* on adult bee and brood haemolymph injures the bees physically, reduces their protein content and wet and dry body weights, and interferes with organ development (Bowen-Walker *et al.*, 1999) [1].

The parasitic mite and the viral diseases they transmit contribute to morphological deformities (small body size, shortened abdomen, deformed wings), which reduce the vigour, longevity and influence the flight duration and homing ability of foragers (Garedew *et al.*, 2004; Kralj and Fuchs, 2006) [6]. The mite weakens the bee's immune system, suppressing the expression of immune-related genes and increasing DWV viral titers, both of which reduce the worker survivorship and colony fitness (Yang and Cox-Foster, 2005; Yang and Cox-Foster, 2007) [16, 15]. Mathialagan *et al.* (2017) [12] reported that *V. jacobsoni* and varroosis are common in Indian bees, *Apis cerana* Fabricius (Hymenoptera: Apidae) in most districts of Tamil Nadu, including Tiruchirappalli and that balanced nutrition coupled with surveillance and management of *V. jacobsoni* are vital to arrest the decline of bee colonies. However, only a few reports are available on the population dynamics of *V. jacobsoni* and its management in India. *Varroa* mites are managed by constant monitoring and timely implementation of various prevention and control methods, *viz*, cultural, physical, chemical and botanicals**.**

Dusting the bees with various dust materials and use of screened bottom board are some of the physical methods to reduce or eliminate the use of synthetic acaricides, which are known to cause resistance in mites as well as residue in the hive and their products. Notable among them are sugar powder dusts (Gregorc *et al.*, 2017) [7], impeding the locomotion of mite (Ramirez, 1994) [14] and stimulating grooming behaviour of honey bees (Macedo *et al.*, 2002) [11]. Dusting has no adverse effect on the capped brood and adult bees in colonies even though bees with

misshapen wings and malformed body walked out and fell down from flight board after dusting (Fakhimzadeh, 2000) [4]. The use of a screen bottom floor was another physical method instead of solid bottom floor to reduce the population growth of *Varroa* by preventing the mites from reinfestation (Ellis *et al.*, 2001; Harbo and Harris, 2004) [2, 8]. Brood-wise, colonies with open screen floor had lower mite population in the brood cells and increased number of capped broods than the wooden floor (Harbo and Harris, 2004) [8]. Hence, the combined effect of screened bottom board and sugar powder dust was studied.

Materials and Methods

The present investigations were carried out in Bee Garden at ADAC & RI, Tiruchirappalli (Lattitude: 10.45ºN; Longitude: 78.36ºE; Altitude: 85 M (MSL) during 2017-18. There were two sets of experimental bee colonies with uniformly aged queens maintained in wooden hives. One set provided with an extra metal screened bottom floor (108 perforations/ sq. inch), coupled with weekly application of powdered sugar as dusting over the bees at the rate of 3- 4 g per frame of bees. The other set was without a screened bottom and powdered sugar dusting. Both sets of hives were provided with a laminated white sheet over the solid wooden bottom board in order to facilitate easier mite counts. Each set consisted of 10 hives.

Assessment of *V. jacobsoni* in *A. cerana*

Populations of *V. jocobsoni* as assessed by examining the accumulated debris on the bottom board, brood in the brood comb, and worker/drone bees.

Debris examination: In the debris examination method, the number of adult *V. jocobsoni* mites that were found dead or alive on the laminated white sheet placed on the wooden bottom board was recorded at weekly intervals from a 250 cm^2area using a transparent 1-cm grid.

Brood examination: In the brood examination method performed at fortnightly interval, the sealed pupae, preferably that of drones, were collected by excising 50 cm^2 comb sample with a sharp alcohol-sterilized knife from a suitable brood comb in each hive. The comb samples were decapped to collect the larva, prepupa or pupa from the cells for examination under a stereo zoom microscope (CETI - Medline Scientific).The presence of small white spots (excreta) indicated a mite-infested cell, in which counts were made on adult mite, separately for workers and drones.

Adult bee examination: In the adult bee examination method, ca. 50 adult worker bees were transferred alive to a 400 ml capacity PET jar containing powdered table sugar (20 g) and covered with a wire mesh screen lid. Drones were collected individually from the hive using a pair of blunt forceps, while workers were collected by shaking the bees off a comb directly into the open jar after ensuring that there was no queen/drones on the comb. If the queen was present, the next frame was selected for dislodging the bees in the concerned experimental hive. After closing the jar with the screen-lid, the mites were dislodged by shaking the bee-filled jar vigorously, coupled with rolling from side to side. Then the dust particles were emptied into water in a whitish autoclavable Petri dish to count the dislodged mites after the powdered sugar got dissolved. The dust-laden bees in jars were then quickly released back in to the hive. Care was taken to ensure that the queen was not captured during the investigation.

The data on debris examination, adult bee examination and brood examination were analyzed after $\sqrt{(x+0.5)}$ transformation and the means were subjected to paired t- test analyses with Microsoft Excel.

Result and Discussion

The results of the experiment showed that sugar powder dusting caused significantly (P = 0.0095) more mites to fall on the solid bottom board through the open screen (7.01 ± 0.56/250 cm^2) than in control hives (0.63 ± 0.12 /250 cm^2) (Table 1, Fig. 1).The peak in fall was recorded during April, 2018 both in control hives (1.00 ± 0.14 /250 cm^2) and treated hives (13.08 ± 1.44 / 250 cm^2). The fallen mites were much less during March, 2018 in control hives (0.43 ± 0.13 / 250 cm^2) and during February, 2018 in treated hives (3.85 ± 0.45 / 250 cm^2).Although there was no significant difference between the treated hives (1.45 ± 0.24 / hive) and control hives (1.06 ± 0.18 / hive) in the mean number of *Varroa* mites found on drones, the population in treatment hives was more than that in control hives, except in February, 2018 (Table 2, Fig. 2). *Varroa* mites attached on to the worker bees showed no significant difference in numbers between the control hives (0.20 ± 0.04 / 50 bees) and treatment hives (0.25 ± 0.05 / 50 bees) following dusting with powdered sugar and the open screen in place (Table 3, Fig. 3). However, more mites were found in treated hives (0.40 ± 0.12 / 50 bees) during April, 2018 and in control (0.35 ± 0.07 / 50 bees) during February, 2018.

The brood examination results indicated no significant difference between control hives (2.39 ± 0.66 / 50 cm^2 brood area) and treatment hives (3.93 ± 0.83 / 50 cm^2 brood area) in the overall number of *Varroa* nymphs found in drone cells (Table 4, Fig. 4).There was no significant difference in the overall number of *Varroa* mites in drone cells between treatment hives (1.49 ± 0.43 / 50 cm^2 brood area) and control hives (1.19 ± 0.27 / 50 cm^2 brood area). However, at the time of fortnightly observations, they were more in treatment hives than in control hives, except the second fortnight of January, 2018 and first fortnight of February, 2018 (Table 5, Fig. 5). There were no eggs and nymphs in capped worker cells. The number of adult *Varroa* mites in capped worker cellsexhibited no significant difference between control hives (0.14 ± 0.08 / 50 cm^2 brood area) and treatment hives (0.28 ± 0.05 / 50 cm^2 brood area) (Table 6, Fig. 6). However, more mites were found in treatment hives than in control hives, except second fortnight of March, 2018.

The results of the experiments revealed that sugar powder dusting cause significantly more number of phoretic mites to fall from the adult bees (Fakhimzadeh, 2000) [4] and the hives equipped with screened bottom showed more number of bottom board mite count than in solid wooden bottom board. It might be due to removal of dead mite by adult bees or refinfestation of fallen alive mite again into the bees on wooden bottom board and both way of mite removal were arrested in the hive with screened bottom board (Ellis *et al.*, 2001; Harbo and Harris, 2004) [2, 8]. The brood and adult bee examination did not yield satisfactory result, where the sugar dusting can not interrupt the mites reproduction in the capped brood cell (Ellis, 2009) [3] and it implies that the repeated load of phoretic mite on adult bees emerged from the infected cells. Finally, dusting powdered sugar combined with screened bottom significantly affect the phoretic mite population but not the reproductive mites and nymphs present

in capped brood cells. Hence, combined use of screened bottom board and sugar powder dusting was only effective when it was incorporated with the integrated management practice of *Varroa* mite and sugar dusting ensures very good mite control by dusting on bees during brood less season.

Table 1: Effect of screened bottom and sugar powder dusting on *V. jacobsoni* on bottom board

Month	Fallen mites/250 cm^2 area of bottom board (no.)		
	Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	0.55 ± 0.13 (0.94)	7.48 ± 1.40 (2.05)	2.95 (P = 0.005)
January, 2018	0.65 ± 0.19 (0.99)	5.45 ± 0.84 (1.74)	2.38 (P =0.013)
February, 2018	0.53 ± 0.13 (0.99)	3.85± 0.45 (1.72)	2.29 (P = 0.022)
March, 2018	0.43 ± 0.13 (0.93)	5.18 ± 0.87 (1.96)	2.89 (P = 0.008)
April, 2018	1.00 ± 0.14 (2.84)	13.08 ± 1.44 (1.15)	2.12 (P = 0.03)
Mean	0.63 ± 0.12 (1.00)	7.01 ± 0.56 (2.06)	2.79 (P = 0.0095)

Mean ± SE; Figures in parentheses are square root+ 0.5transformed values; Mean of 10 observations

Table 2: Effect of screened bottom and sugar powder dusting on *V. jacobsoni* found on drones of *A. cerana*

Month	Mites on drones / Bee hive (no.)		
	Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	0.23 ± 0.13 (0.790	0.35 ± 0.19 (0.86)	NS
January, 2018	0.58 ± 0.18 (0.95)	1.35 ± 0.50 (1.13)	NS
February, 2018	2.18 ± 0.53 (1.30)	1.78 ± 0.58 (1.27)	NS
March, 2018	0.80 ± 0.24 (1.03)	1.48 ± 0.51 (1.22)	NS
April, 2018	1.50 ± 0.37 (1.26)	2.30 ± 0.43 (1.38)	NS
Mean	1.06 ± 0.18 (1.07)	1.45 ± 0.24 (1.17)	NS

Mean ± SE; Figures in parentheses are square root+ 0.5 transformed values; Mean of 10 observations

Table 3: Effect of screened bottom and sugar powder dusting on *V. jacobsoni* found on workers of *A. cerana*

Month	Mites / 50 worker bees (no.)		
	Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	0.10 ± 0.08 (0.72)	0.23 ± 0.10 (0.79)	NS
January, 2018	0.20 ± 0.10 (0.79)	0.28 ± 0.09 (0.84)	NS
February, 2018	0.35 ± 0.07 (0.88)	0.20 ± 0.06 (0.83)	NS
March, 2018	0.20 ± 0.06 (0.80)	0.13 ± 0.08 (0.76)	NS
April, 2018	0.15 ± 0.06 (0.78)	0.40 ± 0.12 (0.91)	NS
Mean	0.20 ± 0.04 (0.80)	0.25 ± 0.05 (0.83)	NS

Mean ± SE; Figures in parentheses are square root+ 0.5 transformed values; Mean of 10 observations

Table 4: Effect of screened bottom and sugar powder dusting on *Varroa* nymphs in drone cells

Month	Fortnightly Count	*Varroa* nymphs in drone cells / 50 cm^2 comb (no.)		
		Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	I count	0.10 ± 0.10 (0.76)	1.70 ± 1.05 (1.05)	NS
	II count	0.10 ± 0.10 (0.76)	2.80 ± 1.44 (1.32)	NS
January, 2018	I count	0.10 ± 0.10 (0.76)	1.20 ± 0.66 (1.06)	NS
	II count	0.40 ± 0.27 (0.85)	0.10 ± 0.10 (0.76)	NS
February, 2018	I count	6.30 ± 2.91 (1.82)	1.00 ± 0.42 (1.07)	NS
	II count	1.70 ± 1.05 (1.05)	5.80 ± 3.09 (1.69)	NS
March, 2018	I count	0.50 ± 0.27 (0.90)	1.50 ± 0.81 (1.13)	NS
	II count	10.10 ± 5.02 (2.12)	6.20 ± 2.66 (1.92)	NS
April, 2018	I count	2.70 ± 1.26 (1.40)	16.10 ± 9.84 (2.05)	NS
	II count	1.90 ± 0.74 (1.29)	2.90 ± 1.66 (1.32)	NS
Mean		2.39 ± 0.66 (1.17)	3.93 ± 0.83 (1.34)	NS

Mean ± SE; Figures in parentheses are square root+ 0.5 transformed values; Mean of 10 observations

Table 5: Effect of screened bottom and sugar powder dusting on adult *Varroa* mite in drone cells

Month	Fortnightly Count	*Varroa* adults in drone cells / 50 cm^2 comb (no.)		
		Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	I count	0.30 ± 0.15 (0.82)	2.10 ± 0.90 (1.29)	NS
	II count	0.10 ± 0.10 (0.76)	2.10 ± 1.07(1.22)	NS
January, 2018	I count	0.10 ± 0.10 (0.76)	1.90 ± 0.99 (1.20)	NS
	II count	1.60 ± 0.62 (1.16)	0.20 ± 0.13 (0.79)	NS
February, 2018	I count	8.50 ± 5.32 (2.13)	2.00 ± 1.08 (1.34)	NS
	II count	1.30 ± 0.54 (1.09)	3.60 ± 1.25 (1.42)	NS
March, 2018	I count	0.80 ± 0.29 (1.01)	4.50 ± 1.66 (1.77)	NS
	II count	4.40 ± 1.83 (1.68)	6.90 ± 3.16 (1.95)	NS
April, 2018	I count	1.40 ± 0.71 (1.19)	9.70 ± 6.27 (2.05)	NS
	II count	2.00 ± 0.68(1.31)	5.40 ± 1.83 (1.85)	NS
Mean		2.05 ± 0.45 (1.19)	3.84 ± 0.80 (1.49)	NS

Mean ± SE; Figures in parentheses are square root+ 0.5 transformed values; Mean of 10 observations

Table 6: Effect of screened bottom and sugar powder dusting on adult *Varroa* mite in worker cells

Month	Fortnightly Count	*Varroa* adults in worker cells / 50 cm^2 comb (no.)		
		Without screened bottom and sugar powder dust (control)	Screened bottom + sugar powder dust (treatment)	*t* - value
December, 2017	I count	0.10 ± 0.10 (0.76)	0.20 ± 0.13 (0.81)	NS
	II count	0.10 ± 0.10 (0.76)	0.60 ± 0.27 (0.97)	NS
January, 2018	I count	0.10 ± 0.10 (0.76)	0.20 ± 0.13 (0.81)	NS
	II count	0.10 ± 0.10 (0.76)	0.80 ± 0.33 (0.93)	NS
February, 2018	I count	0.10 ± 0.10 (0.76)	0.20 ± 0.13 (0.81)	NS
	II count	0.30 ± 0.15 (0.82)	0.30 ± 0.15 (0.86)	NS
March, 2018	I count	0.20 ± 0.13 (0.79)	0.20 ± 0.13 (0.79)	NS
	II count	0.20 ± 0.13 (0.81)	0.10 ± 0.10 (0.76)	NS
April, 2018	I count	0.10 ± 0.10 (0.76)	0.10 ± 0.10 (0.76)	NS
	II count	0.10 ± 0.10 (0.76)	0.10 ± 0.10 (0.76)	NS
Mean		0.14 ± 0.08 (0.77)	0.28 ± 0.05 (0.83)	NS

Mean ± SE; Figures in parentheses are square root+ 0.5 transformed values; Mean of 10 observations

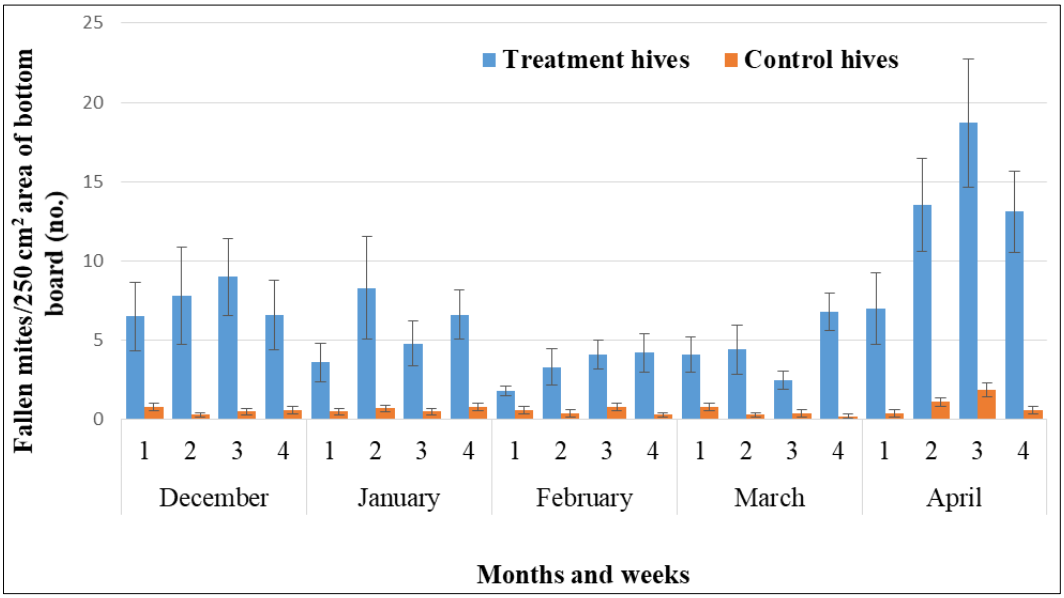

Fig 1: Effect of screened bottom and sugar powder dusting on *Varroa* mites fallen on the bottom board (Vertical bars indicate the standard error. Mean of 10 observations).

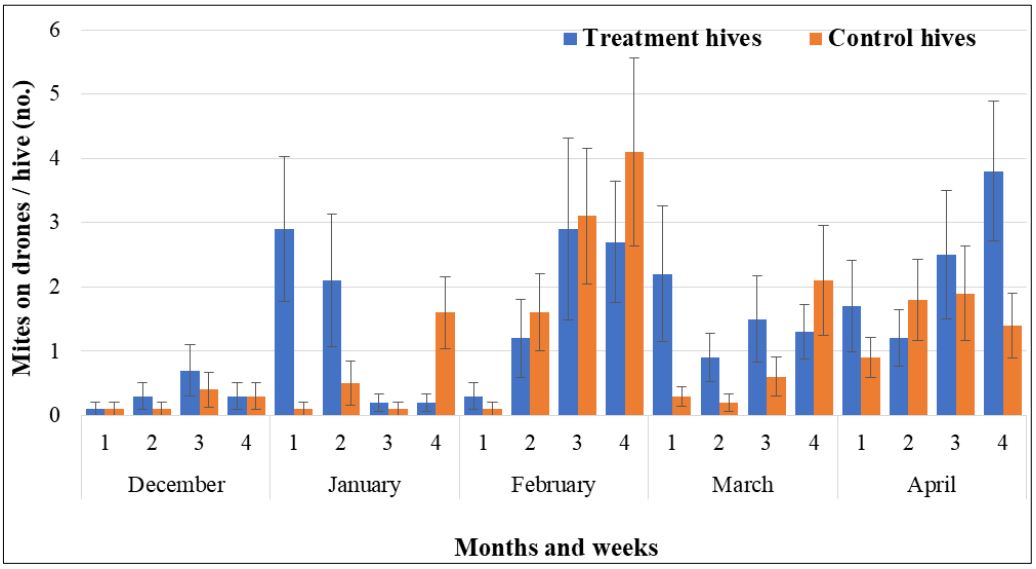

Fig 2: Effect of screened bottom and sugar powder dusting on *Varroa* mites on drones of *A. cerana.* (Vertical bars indicate the standard error. Mean of 10 observations).

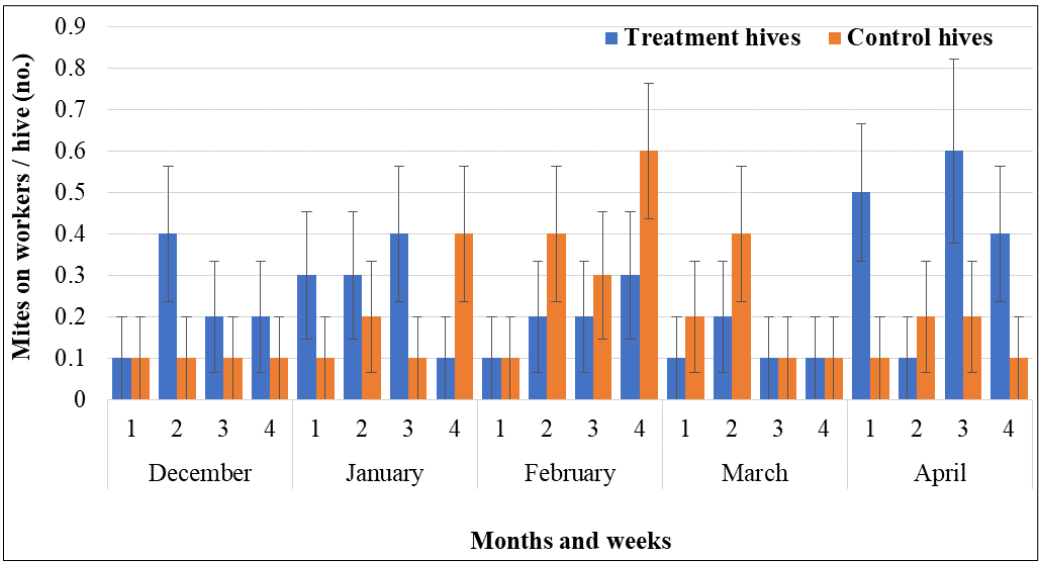

Fig 3: Effect of screened bottom and sugar powder dusting on *Varroa* mites on workers of *A. cerana* (Vertical bars indicate the standard error. Mean of 10 observations).

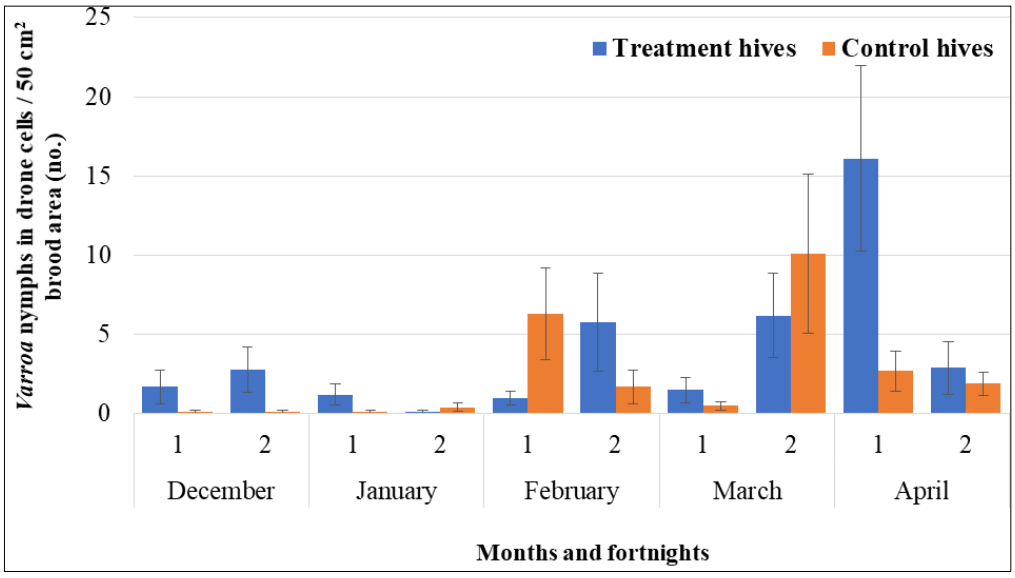

Fig 4: Effect of screened bottom and sugar powder dusting on *Varroa* nymphs in *A. cerana* drone cells. (Vertical bars indicate the standard error. Mean of 10 observations).

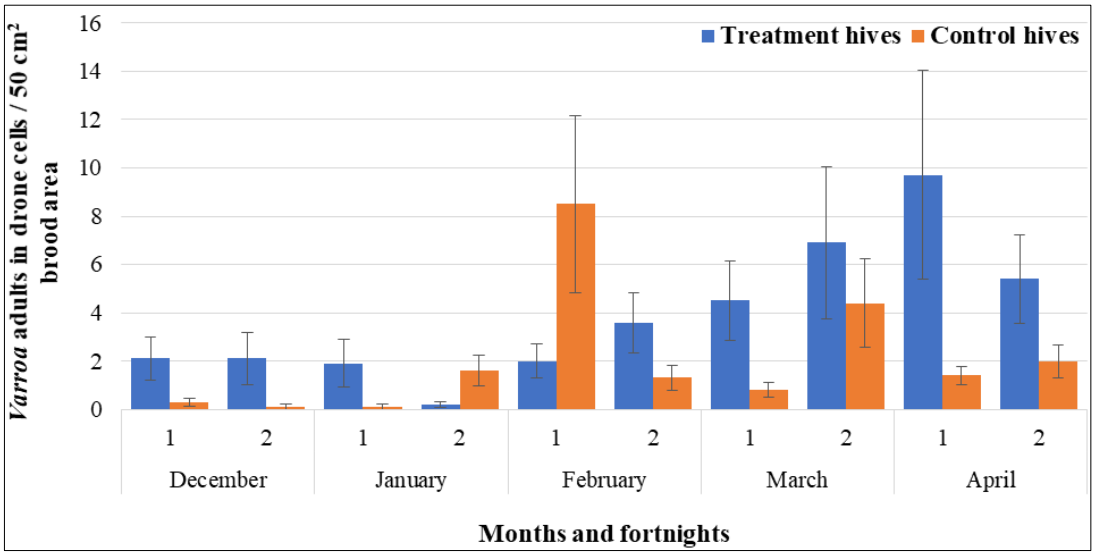

Fig 5: Effect of screened bottom and sugar powder dusting on *Varroa* adults in *A. cerana* drone cells. (Vertical bars indicate the standard error. Mean of 10 observations).

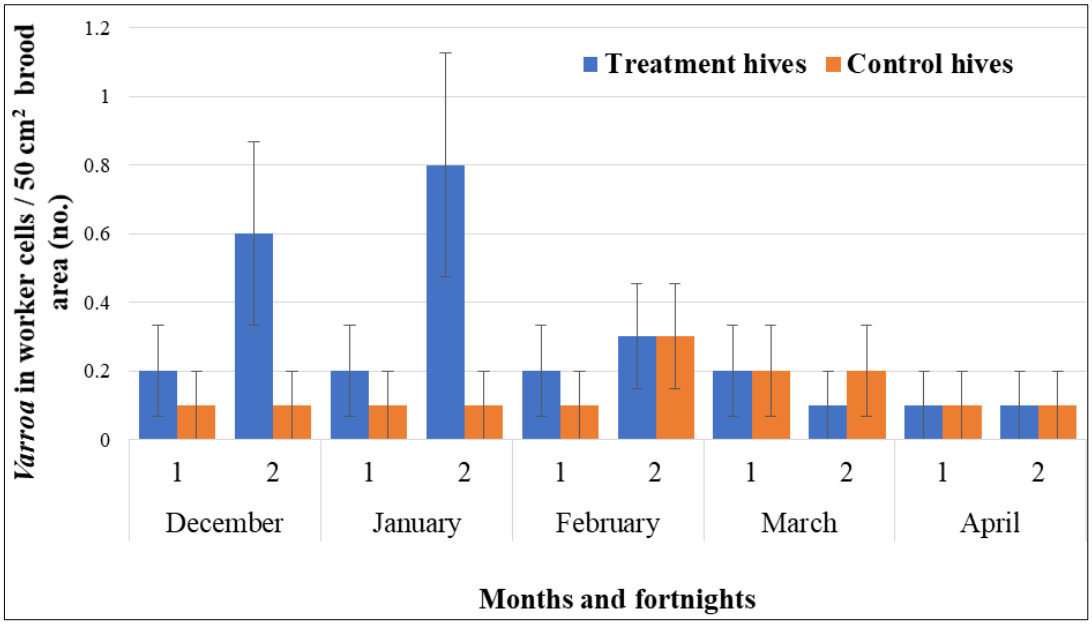

Fig 6: Effect of screened bottom and sugar powder dusting on *Varroa* adult mites in worker cells. Vertical bars indicate the standard error. Mean of 10 observations.

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