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Research article
Ερευνητικό άρθρο

Effect of collecting bee venom on the defensive behaviour of *Apis mellifera*

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ABSTRACT: Bees tend to sting the predator to defend their resources. In this action, alarm pheromone is also released which affect many colony activities. To determine the influence of weekly bee venom collection on the defensive behaviour of *Apis mellifera* Linnaeus colonies, experiments were conducted using two types of bee venom collectors (DPS-BVC-01 and Bee Whisper 5.0) on two bee strengths (8 and 16 bee-frames) during four seasons (monsoon, autumn, spring and summer) at *Apis mellifera* Apiary of Punjab Agricultural University, Ludhiana (India). Defensive behaviour of the colonies was assessed by swinging suede leather wrapped black leather ball in front of the colony entrance. One day after venom collection, defensive activity of the colonies (number of stings received on black leather ball per min) increased by 9.66 per cent compared to number of stings received one day before the venom collection. It decreased thereafter, and become on par with pre-venom collection status after three days of venom collection. Among all the four seasons, the highest defensive response was observed during the summer season followed by in monsoon, spring and autumn seasons. During all the seasons, 16 bee-frame strength colonies stung 37.42 per cent more than 8 bee-frame strength colonies. Further, the colonies exposed to DPS-BVC-01 (9 V) were 33.08 per cent more defensive than colonies exposed to Bee Whisper 5.0 (3 V) bee venom collector. However, exposure period (30 and 60 min) to venom collector did not show any significant difference in influencing the defensive behaviour of the honey bee colonies, thus venom can be safely collected from colonies.

Keyword: Aggressiveness; *Apis mellifera*; bee strength; defensive behaviour; venom collection

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INTRODUCTION

Honey bees are one of the most valuable beneficial insect taxa present on Earth. Besides rendering pollination services, bees also provide nutritional, medicinal, and industrial products like honey, royal jelly, pollen, bees wax, propolis, and bee venom. Among these, bee venom is a premium product. Bee venom is produced by female honey bees and has long been finding use in the pharmaceutical as well as in the cosmetic industries. Female auxiliary reproductive glands give rise to the epidermal glands that produce bee venom. A honey bee venom gland is a simple, long, thin, distally bifurcated structure that opens into an ovoid reservoir, also known as venom sac (Bridges and Owen, 1984). Venom production gradually rises over the first two weeks of an adult worker's emergence and is at peak when the worker bees started performing the duties of hive defence and foraging, usually after two to three weeks of emergence following which it decreases as the bee ages. In queen bee, venom production has been reported to be the highest at emergence, most likely because they must prepare for imminent conflicts with rival queens (Krell, 1996).

Honey bee venom is a bitter colourless and odourless liquid having pH in the range of 4.5 to 5.5 which later dries due to loss of volatile components during collection. The colour of the bee venom is crystalline white which changes to pale yellow due to oxidation on exposure to sunlight (Krell, 1996). It contains at least 18 pharmacologically active components. It is the complex mixture of active peptides (melittin, adolapin, apamin, MCD, secapin, pamin, minimin, etc.), enzymes (phospholipase A2 and B, hyaluronidase, phosphatase, and α-glucosidase), biogenic amines (histamine, dopamine, noradrenalin), amino acids (aminobutyric acid, α-amino acids), phospholipids, sugars (glucose and fructose), volatiles (pheromones), minerals (P, Ca, Mg) and other components (Bogdanov, 2016).

Being eusocial insects, specialized worker honey bees called guard bees (only 10-15 % of adult worker bees) have evolved a coordinated defensive reaction to ensure colony survival and protect their colony resources (food stores in the form of honey and pollen, as well as the brood, the queen and the bees themselves) against a wide range of predators and parasites (Nouvian *et al.,* 2016; Gage *et al.,* 2018). They use the alarm pheromone to convey massage to hive bees to initiate colony defense by using bee venom as a defense tool. It is a complex trait that is influenced by many factors like honey bee race, seasonal and weather factors, colony strength and health, time of the day, and foraging activity (Omar, 2020).

Pheromone secretion is thought to be one of the primary cues for initiating aggressive behaviour in defending worker bees (Dotimas and Hider, 1987). By releasing alarm pheromones, they can recruit other bees to help them handle large predators. These chemicals trigger both rapid and longer-term changes in the behaviour of nearby bees, thus priming them for defence (Nouvian *et al.,* 2016). The major components of alarm pheromone secreted from the Koshevnikov gland include isopentyl acetate (elicits stinging to encounter intruders, attracts other nest mates to join in defence, and repels foragers) and an oil-like component, (Z)-11-eicosen-1-ol which trigger stinging. Some other components of alarm pheromone are 1-hexanol, butyl acetate, octyl acetate, 1-butanol, hexyl acetate, 1-octanol, and 2-nonanol which may not elicit stinging but could help to recruit other nestmates to attend to defence activity. Other than the secretions of Koshevnikov gland, mandibular gland's secretion of 2-heptanone also elicit the alarming activity of honey bees (Wang and Tan, 2019).

Earlier, the venom was collected manually by surgically removing the venom sac which was more laborious and yielded only a little quantity. But later, it was replaced by electric shock method in which honey bees are stimulated through mild electric shock to sting on a glass plate from which bee venom is collected through scrapping upon drying. A large quantity of alarm pheromones is also released during the process that alter the communication, behaviour or physiology of honey bees (Dotimas and Hider, 1987; Bovi *et al.*, 2017; Modanesi *et al.,* 2015; Onari *et al.,* 2016). Mean larval brood survival also reported to decrease by 6.08-6.30 per cent in the colonies exposed to bee venom collector during monsoon season (Sidana et al., 2022). Hygienic behaviour , is a character owned by honey bees to defend *Varroa destructor* Anderson and Trueman infestation (Bharathi et al., 2020). This means a colony having high hygienic behaviour will have lesser brood menaces. Though it is a genetically governed character yet there is a report in which it get enhanced after installing a bee venom collection device (El-Saeady et al., 2016).

Therefore, this study was conducted to determine the extent to which installation of bee venom collec-

tion device affect on the defensive behaviour of *A. mellifera* colonies.

MATERIAL AND METHODS

The experiment was carried out during four seasons (monsoon and autumn in 2021; spring and summer in 2022) at *Apis mellifera* Apiary, Entomological Research Farm, Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India (30.9041° N, 75.8066° E). To study the effect of venom collection on the defensive behaviour of honey bees, a total of 24 colonies of *Apis mellifera ligustica* Spinola were selected. These colonies were divided into two sets having bee strengths of 8 and 16 bee-frames. The colonies were exposed for 60 and 30 min.

Two type of bee venom collectors (DPS-BVC-01, Mfg. M/s DPS Tech. Smart Private Limited, New Delhi, India and Bee Whisper 5.0, Mfg. M/s IGK Electronics Limited, Bulgaria) were tested in this study. The bee colonies were pre-equalized with respect to normal brood area availability (uncapped and capped), and food stores (pollen and honey) for each of the two strength colonies. The colonies were inspected regularly and management strategies (formic acid) against bee parasitic mites were applied uniformly to all the colonies including control group. The following were the treatments which were classified into eight different experimental groups, each having three replications:

T_1 : 8 bee-frame strength colonies exposed to DPS-BVC-01 for 30 min

T_2 : 8 bee-frame strength colonies exposed to DPS-BVC-01 for 60 min

T_3 : 8 bee-frame strength colonies exposed to Bee Whisper 5.0 for 30 min

T_4 : 8 bee-frame strength colonies exposed to Bee Whisper 5.0 for 60 min

T_5 : 16 bee-frame strength colonies exposed to DPS-BVC-01 for 30 min

T_6 : 16 bee-frame strength colonies exposed to DPS-BVC-01 for 60 min

T_7 : 16 bee-frame strength colonies exposed to Bee Whisper 5.0 for 30 min

T_8 : 16 bee-frame strength colonies exposed to Bee Whisper 5.0 for 60 min

Bee venom collection

For the collection of honey bee venom, the bee venom collection apparatus was installed horizontally on wooden board (25.40 x 45.72 cm) in front of the hive entrance and turned on for 30 or 60 minutes during the evening hours (1600-1700 h) in all the four seasons. DPS-BVC-01 bee venom collector has larger dimensions of wire grid (13 x 26.5 cm) and works on 9 V battery compared to 13 x 20.5 cm grid dimensions in Bee Whisper 5.0 venom collector powered by two AA batteries of 1.5 V each (Plate 1 a & b). After the exposure period, apparatus was turned off and glass plate was removed from the apparatus and, dried bee venom was collected from glass surface. The process was repeated six times at weekly interval in every season.

a b

Plate 1. Bee venom collectors (a) DPS-BVC-01 and (b) Bee Whisper 5.0 installed on *Apis mellifera* colony.

Defensive response of the honey bees

The defensive behaviour of the *A. mellifera* colonies was assessed one day before, and one and three days after the venom collection during all the four seasons. A rubber ball of (diameter 6 cm) was wrapped in black suede leather of length x breadth (22 x 20 cm) which was attached to the end of a one-metre long metallic wire (Plate 2). The suede leather was charged with 100 µl alarm pheromone (98 per cent isopentyl acetate) each time just before use. The ball was oscillated manually in a rhythmic way approximately at 10 cm distance in front of the hive entrance. The ball was oscillated exactly for 60 seconds for recording the number of stings received per minute. A fresh ball covered with fresh suede leather was used for each colony to prevent the accumulation of alarm pheromone from treated balls. After completion of test on a given colony, the ball was placed inside a plastic bag to count the stings later in the laboratory. After counting, the stings were removed from the ball with a forceps and the ball was left in sun for two hours so that the alarm pheromone residue left on the leather get evaporated.

Statistical analysis

Data were statistically analysed for Analysis of Variance (ANOVA) following factorial Completely Randomised Design (CRD) to test the significance of differences among various treatment means using CPCS1 software. The data were prior subjected to numerical $\sqrt{n+1}$ transformation before statistical analyses. The Least Significant Difference (LSD) was used to compare the means at 5 per cent level of significance.

RESULTS

Stinging instinct i.e. defensive behaviour of the *A. mellifera* colonies varied significantly with respect to the bee venom collector used, seasons of venom collection and bee strengths of colonies. Overall, the defensive response of the colonies increased significantly one day after the venom collection (13.05 ± 0.25 stings/min/colony) compared to one day before the venom collection (11.90 ± 0.22 stings/min/colony) (Table 1). However, the mean number of stings received on black leather ball decreased significantly to 12.16 ± 0.24 stings/min/colony after three days of venom collection and become on par with pre venom collection status. This trend in stinging behaviour was observed during all the four seasons. However, the stinging response of the colonies to bee venom collection varied significantly among the various seasons also (Figure 1), being the highest during summer (17.17stings/min/colony) followed by in monsoon (15.15stings/min/colony), spring (11.08stings/min/colony) and autumn seasons (8.77stings/min/colony) after one day of venom collection.

Further, among the two bee strengths used, 16 bee-frame strength *A. mellifera* colonies exposed

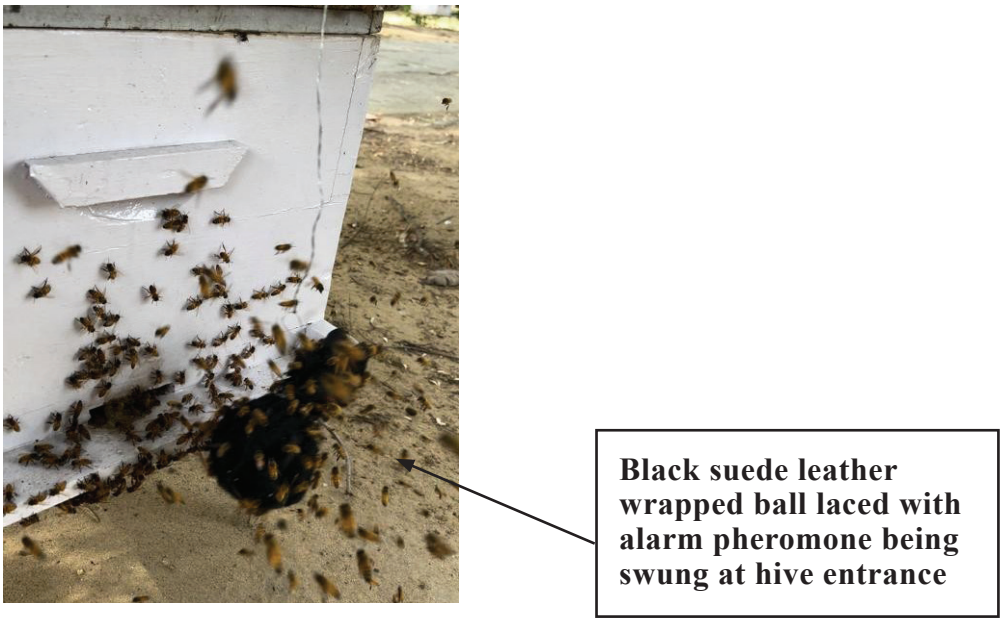

Plate 2. Alarm pheromone assay being conducted on *Apis mellifera* colony to assess defensive behaviour.

Table 1. Effect of bee venom collector, exposure period and colony strength on defensive behaviour of *A. mellifera* colonies

Treatments (Type of venom collector and exposure period)		[3]Mean number of stings (± SE) received on black leather ball per min										
		8 Bee-frame strength					16 Bee-frame strength					Pooled mean (Equipment x Exposure period)
		1 DBT[4]	1 DAT[5]	3 DAT	Mean	Pooled mean (Strength x Exposure period)	1 DBT	1 DAT	3 DAT	Mean	Pooled mean (Strength x Exposure period)	
[1]DPS-BVC-01 (9 V) venom collector exposed for 30 min		10.78 ± 0.24 (3.43)	12.46 ± 0.37 (3.67)	11.06 ± 0.19 (3.47)	11.43 ± 0.27 (3.53)	10.26±0.24 (3.36)	15.03 ± 0.19 (4.00)	17.65 ± 0.16 (4.32)	15.83 ± 0.23 (4.10)	16.17 ± 0.20 (4.14)	14.29 ± 0.26 (3.91)	13.80 ±0.23 (3.85)
[2]Bee Whisper 5.0 (3 V)venom collector exposed for 30 min		8.90 ± 0.10 (3.15)	9.27 ± 0.33 (3.20)	9.09 ± 0.18 (3.18)	9.09 ± 0.20 (3.18)		12.14 ± 0.32 (3.62)	12.54 ± 0.13 (3.68)	12.56 ± 0.51 (3.68)	12.41 ± 0.32 (3.66)		10.75 ± 0.26 (3.43)
DPS-BVC-01 venom collector exposed for 60 min		11.39 ± 0.30 (3.52)	13.36 ± 0.20 (3.79)	11.92 ± 0.25 (3.59)	12.22 ± 0.25 (3.64)	10.58±0.23 (3.40)	15.61 ± 0.20 (4.08)	18.14 ± 0.31 (4.37)	16.21 ± 0.04 (4.15)	16.65 ± 0.18 (4.20)	14.34 ± 0.24 (3.92)	14.44 ± 0.22 (3.93)
Bee Whisper 5.0venom collector exposed for 60 min		9.04 ± 0.13 (3.17)	8.82 ± 0.21 (3.13)	8.92 ± 0.26 (3.15)	8.93 ± 0.20 (3.15)		12.28 ± 0.28 (3.64)	12.13 ± 0.29 (3.62)	11.70 ± 0.30 (3.56)	12.03 ± 0.29 (3.61)		10.48 ± 0.24 (3.39)
Mean (DPS-BVC-01 venom collector)		11.09 ± 0.27 (3.48)	12.91 ± 0.29 (3.73)	11.49 ± 0.22 (3.53)	11.83 ± 0.26 (3.58)		15.32 ± 0.19 (4.04)	17.89 ± 0.24 (4.35)	16.02 ± 0.14 (4.13)	16.41 ± 0.19 (4.17)		
Mean (Bee Whisper 5.0 venom collector)		8.97 ± 0.12 (3.16)	9.04 ± 0.27 (3.17)	9.00 ± 0.22 (3.16)	9.01 ± 0.20 (3.16)		12.21 ± 0.30 (3.63)	12.34 ± 0.21 (3.65)	12.13 ± 0.40 (3.62)	12.22 ± 0.30 (3.64)		
Pooled mean (Day x Strength)		10.03 ± 0.19 (3.32)	10.98 ± 0.28 (3.46)	10.25 ± 0.22 (3.35)	10.42 ± 0.23 (3.38)		13.77 ± 0.25 (3.84)	15.11 ± 0.22 (4.01)	14.07 ± 0.27 (3.88)	14.32 ± 0.25 (3.91)		
Pooled mean (Day)		11.90 ± 0.22 (3.59)	13.05 ± 0.25 (3.75)	12.16 ± 0.24 (3.63)	12.37 ± 0.24 (3.66)							
Pooled mean (Equipment x Day)	**DPS-BVC-01**	13.20 ± 0.23 (3.77)	15.40 ± 0.26 (4.05)	13.76 ± 0.18 (3.84)	14.12 ± 0.22 (3.89)							
	Bee Whisper 5.0	10.59 ± 0.21 (3.40)	10.69 ± 0.24 (3.42)	10.56 ± 0.31 (3.40)	10.61 ± 0.25 (3.41)							
Pooled mean (Exposure period x Day)	**30 min**	11.71 ± 0.21 (3.57)	12.98 ± 0.25 (3.74)	12.13 ± 0.28 (3.62)	12.28± 0.25 (3.64)							
	60 min	12.08 ± 0.23 (3.62)	13.11 ± 0.25 (3.76)	12.19 ± 0.21 (3.63)	12.46 ± 0.23 (3.67)							
LSD (p = 0.05)		Equipment = (0.03), Day = (0.04), Exposure period = NS, Strength = (0.03), Equipment x Day = (0.05), Equipment x Exposure period = (0.04), Equipment x Strength = (0.04), Day x Exposure period = NS, Day x Strength = NS, Exposure period x Strength = NS, Equipment x Day x Exposure period = NS, Equipment x Day x Strength = NS, Equipment x Exposure period x Strength = NS, Day x Exposure period x Strength = NS and Equipment x Day x Exposure period x Strength = NS										

Figures in parentheses indicate means of $\sqrt{n+1}$ transformation values

[1]DPS-BVC-01 (Indigenous venom collector, 9 volts, Mfg. M/s SB Tech. Smart Private Limited, New Delhi)

[2]Bee Whisper 5.0 (Imported venom collector, 3 volts, Mfg. M/s IGK Electronics Limited, Bulgaria)

[3]Mean of observations of four seasons

[4]Days before treatment

[5]Days after treatment

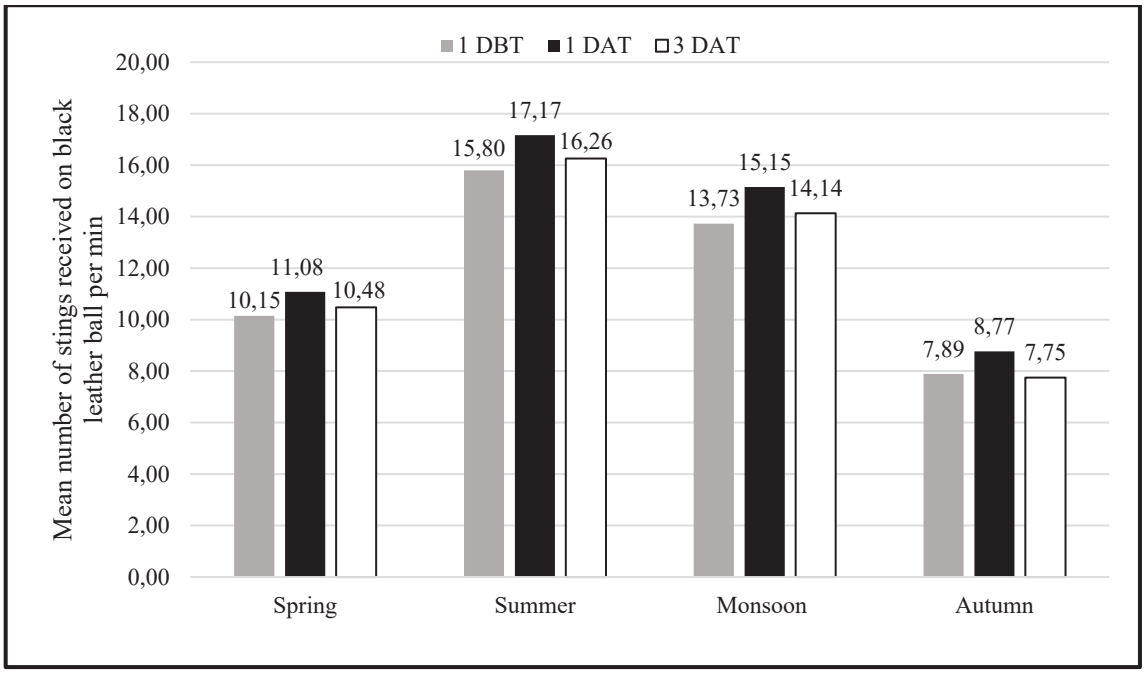

Figure 1. Comparative aggressiveness of *A. mellifera* colonies after venom collection in various seasons.

to venom collection were more defensive (14.32 ± 0.25 stings/min/colony) than 8 bee-frame strength colonies (10.42 ± 0.23 stings/min/colony). Significant difference in the mean number of stings per min was observed between the two bee venom collectors used. The colonies exposed to DPS-BVC-01 (9 V) were significantly more defensive (14.12±0.22 stings/min/colony) than the colonies exposed to Bee Whisper 5.0 (3 V) bee venom collector (10.61±0.25 stings/min/colony). However, there was no significant variation in the defensive behaviour of the colonies between the exposure periods (30 and 60 min).

Regarding the combined interaction between type of equipment used and observation days, significantly the highest mean number of stings (15.40±0.26 stings/min/colony) was recorded from the colonies exposed to DPS-BVC-01 bee venom collector after one day of venom collection followed by colonies exposed to same venom collector after three days of venom collection (13.76 ± 0.18 stings/min/colony). Whereas the colonies which were exposed to Bee Whisper 5.0, there was no significant difference in the defensive behaviour of the colonies among the observation days. The colonies which were exposed to DPS-BVC-01 for 60 min were more defensive (14.44±0.22 stings/min/colony) than the colonies exposed to same equipment for 30 min (13.80±0.23 stings/min/colony). On contrast, the colonies exposed to Bee Whisper 5.0 bee venom collector for 60 min were the least defensive (10.48±0.24 stings/min/colony) and were on par with the colonies exposed to same equipment for 30 min (10.75±0.26 stings/min/colony), and both the latter treatments (employing Bee Whisper 5.0) evolved significantly lesser stinging response than with the former treatments employing DPS-BVC-01 equipment.

Data on interaction between the equipment used and bee strength of the exposed colonies showed that significantly the highest defensive behaviour was shown by 16 bee-frame strength colonies exposed to DPS-BVC-01 bee venom collector (16.41±0.19 stings/min/colony) followed by same strength colonies exposed to Bee Whisper 5.0 (12.22±0.30 stings/min/colony). These two values were significantly higher than the values obtained for 8 bee-frame strength colonies (11.83 ± 0.26 and 9.01 ± 0.20 stings/min/colony, respectively). DPS-BVC-01 resulted in evoking more defensive response in both the colony strengths compared to corresponding response observed using Bee Whisper 5.0. Significantly the least defensive behaviour was shown by 8 bee-frame strength colonies exposed to Bee Whisper 5.0 bee venom collector.

DISCUSSION

There are divergent opinions regarding the effect of venom collection by electric shock method on the defensive behaviour of the honey bee colonies over the globe as there are no universal standards regarding equipment voltage, duration of exposure period, strength of colonies used for venom collection, etc.

Our results revealed that defensive behaviour of honey bee colonies increased significantly one day after the venom collection in both 8 and 16 bee-frame strength *A. mellifera* colonies, though their aggressiveness became on par with the pre-venom collection status after 3 days of venom collection. This increase in defensive response of the colonies might be due to release of large amount of alarm pheromone (López-Incera et al., 2021). The same condition arisen during the process of venom collection process thereby triggering the defensive response in honey bee colonies (Nouvian *et al.*, 2016). Contrary to this, Morse and Benton (1964) had reported that bees remained highly aggressive even after 6-7 days of venom collection and were ready to sting anyone who came within radius of few hundred feet of the apiary. The defensive behaviour diminished at 3 days which has been reported by López-Incera et al. (2021) as an essential strategy adopted by bees to prevent their colonies to get exhausted due to excessive bee loss.

Variation in defensive activity of the colonies among the various seasons might be dependent on several factors like weather condition (temperature, relative humidity, etc.), presence of flora, bee strength, etc. The highest defensive activity of the colonies after venom collection during summer season might be due to higher temperature and shortage of flora during that period. Together, all these factors resulted into significant reduction in foraging activity of the exposed colonies that favoured the larger number of bees available and engaged in the defence of their colony. Zarate et al. (2023) too advocated such behaviour in which limited resources made honey bee colonies to exhibit more defensive behaviour. This ultimately increased the number of stings on black leather ball swung in front of the hive. During monsoon season, although temperature was not that much high as in summer but due to cloudy weather, high humid conditions and scarcity of flora, foraging activity was significantly curtailed and bees were mostly hanging in clusters at hive entrance. These all factors ultimately increased the engagement of bees in defensive activity of the colony. Higher defensive activity of the colonies during summer than monsoon season was favoured by the fact that bee strength of the colonies reduced significantly during monsoon than was in summer season due to scarcity of flora. But during spring and autumn season, weather conditions normalised and also there was plenty of flora outside. This may have resulted into more engagement in foraging and lesser in stinging. Although when comparing spring *vs*. autumn, more defensive activity was observed during spring than autumn which might be due to more growth and higher activity of the colonies during spring than in the autumn season.

Difference in defensive behaviour between the different bee-strength colonies after venom collection might be dependent on the number of bees engaged in the stinging activity. Since in 16 bee-frame strength colonies, there were greater number of bees, and so significantly more number of stings were received than in 8 bee-frame strength colonies. As reported by Collins et al. (1982) larger colonies of *A. mellifera ligustica* inflicted around 12 times more stings than comparatively smaller colonies of 3 bee-frame strength whereas number of bees recruited per minute was around 10 times more.

Between two different collectors used for venom collection, colonies exposed to DPS-BVC-01 were significantly more aggressive than colonies exposed to Bee Whisper 5.0 bee venom collector during all the four seasons. This variation in defensive behaviour of the colonies might be due to the fact that higher quantity of alarm pheromone might have been released during collection by DPS-BVC-01 as it was operated on 3 times higher voltage (9 V) than Bee Whisper 5.0 which was operated on 3 V. Another factor which might also have contributed to the above variation was the grid surface area of the apparatus which in case of DPS-BVC-01 (13 x 26.5 cm), was slightly larger than Bee Whisper 5.0 (13 x 20.5 cm) resulting into excitation of greater number of bees in a given time-framebyDPS-BVC-01 than by Bee Whisper 5.0. Rana et al. (2011) also observed that honey bee colonies exposed to high voltage (26-30 V) stayed disturbed for longer period and needed 5-6 h to return to normal behaviour, compared to colonies exposed to 18-22 V, which normalized in around 2 h. However, in the present study, colonies exposed to much lower voltage (3 V and 9 V) returned to normal defensive status only 3 days after bee

venom collection instead of 2 h reported by Rana et al. (2011).

Our results are in disagreement with the findings of Argena et al. (2021) who reported that bee venom collection did not influence the defensive response of the bees which might be due to relatively gentle behaviour of the Macedonian bee (*Apis mellifera macedonica* Ruttner). There existed a direct relationship between aggressiveness and bee venom collection as reported by Sidana (2022) in which it was reported that the mean quantity of bee venom collection was 11.84, 18.98, 17.52 and 8.27 mg/colony/exposure during spring, summer, monsoon and autumn, respectively. The maximum temperature during corresponding seasons were 30.97, 39.09, 34.02 and 31.50°C, respectively. Also, Modanesi et al. (2015) reported that venom collection for 60 min significantly increased the defensin gene expression, promoted alertness in other worker bees to protect their hive and increased discomfort than 30 min treatment, whereas our study revealed non-significant variation in defensive activity between the colonies exposed for 30 and 60 min.

CONCLUSION

Bee venom collection significantly increased the defensive response in the honey bee colonies which get normalized after three days of venom collection. Summer season accounted for the highest defensive activity followed by monsoon, spring and autumn season. Higher bee strength incited higher stinging response.

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Authors contributions

Sidana V.: Investigation, data curation, writing-first manuscript draft; Choudhary A. writing-review and editing; Singh J.: Conceptualization and methodology refinement; Chhuneja P.K. supervision

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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