



## Research Article

# Hornet pests of honey bees in the Indian Himalayas and a low cost trapping device for their eco-friendly management

Amit Umesh Paschapur, Avupati RNS Subbanna, Manoj Parihar, Sunaullah Bhat, Krishna Kant Mishra, Lakshmi Kant

## Abstract

Honey bees suffer from a cosmic array of biotic and abiotic stress during their life cycle. The insect pests like wax moths and Dermestid beetles contribute immensely to the damage of bee colonies and reduction in honey yield in tropical regions of India. While, the bee colonies in temperate regions of Indian Himalayas, face an entirely different set of hitches that include predation of forager bees by three hornet species (*Vespa mandarina*, *Vespa velutina nigrithorax* and *Vespa tropica*). The attacks on bee colonies by these hornet species compel the bees to desert the colony and swarm away to a new habitat thus, causing severe economic losses to bee keepers. Considering the severe setbacks enforced by hornets, a low-cost hornet trap was designed. The bait ingredients and their trapping efficiency against targeted hornet species are presented herewith. It was observed that green colored bottles attracted 34 hornets of three species in 10 days, while transparent bottles attracted only seven hornets at bait proportions of 1:2:2 (Apple cedar Vinegar: orange juice: water). Moreover, to reduce the attraction of honey bees into the trap, supplementary ingredients like rotten fruits and market honey were used, which enhanced the trapping efficiency of hornets. The benefit-cost ratio of 12.30:1 was recorded after the installation of the trap, which was significantly superior to traditional method (1.64:1) of manual swatting of hornets in the apiary. The farmers are currently trained to design and use these traps for the management of hornet pests in the Indian Himalayas.

**Keywords** bee keeping, honey bees, hornet trap, Indian Himalayas, pest management

## Introduction

Apiculture is an important allied sector of agriculture in India and according to the recent report of the National Bee Board, under the agricultural department, India's honey production in 2017-2018 was 1.05 lakh metric tons (MTs) as compared to the 35,000 metric tons (MTs) in 2005-06 [1]. As per the international demand, the export rate of honey in India has increased by 207% in 2018 and is expected to expand [2]. Moreover, crop pollination by honey bees increased the crop yield manifold. Agricultural experts say that the additional yield obtained due to the pollination by honey bees is 15 to 20 times that of the money generated by stockpile products [3]. Moreover, Uttarakhand has 4,635

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Beekeepers and the state produces around 1,193 metric tonnes of honey per year (2017-18) [4]. Among the major honey-producing districts of Uttarakhand, Haridwar, US Nagar, Nainital and Dehradun top the list [5]. However, beekeeping in the Indian Himalayas has its own set of problems and the major among them are the insect pest infestations [6].

More than 12 insect pests are reported to cause damage to honey bees, hives, and their products in the Indian Himalayas [6]. Although Greater wax moth (*Galleria mellonella*) and lesser wax moth (*Achroia grisella*) are known to cause a threat to apiculture in the Indian Himalayas, recently the hornet pests belonging to the family Vespidae have gained the upper hand and are causing direct damage to the foraging bees and are leading to reduction in colony population, making the colonies weak and finally resulting in absconding or deserting of bee colonies [7]. There are three main hornet species in the state of Uttarakhand that are severe pests in the apiary, viz., giant Asian hornet (*Vespa mandarina*), Yellow-legged hornet (*Vespa velutina nigrithorax*), and Greater banded hornet (*Vespa tropica*). These are semi-social hornets that construct horizontal nests on tall trees, buildings, and in abandoned rodent burrows [8]. The colony population fluctuates between hundreds to thousands, while the peak population is recorded in the monsoon months of August to September in Uttarakhand. Among the three; *V. mandarina* is a notorious and most ferocious species that is observed to kill 120-150 forager bees in an hour [9]. The hornets wait at the hive entrance, catch hold of the foraging bees, decapitate and carry the thoracic part of the bee to their nests to feed the young ones [10]. The hornet damage is very severe in unmanaged apiaries and the foothills, low hills, and mid-hills of the Himalayas, as the climatic conditions are very suitable for the hornet to survive and reproduce [11].

In the mid and low hills of Uttarakhand, the hornets are causing a severe threat to beekeeping. Their attack coincides with the good honey flow period and active foraging of bees, which leads to weak colonies, thus leading to poor honey production and severe economic losses to beekeepers of the region [12]. The traditional management practices like swatting hornets near the bee hives, destruction of nests, hibernating queen, and workers are followed by farmers in the Indian Himalayas [6]. Moreover, few farmers practice fitting entrance reducers to the hive to prevent the entry of hornets into the hive but this does not reduce the predation of forager bees. Martin [13] also recommended the use of specific strains of parasitic nematodes, entomopathogenic fungi, and poison baits for managing the menace of hornets in the apiaries but, these tactics have rarely been successful [14].

Keeping these lacunae in mind, we will briefly elaborate about identification, biology, and predatory Behaviour of three important hornet species of Uttarakhand Himalayas and introduce the farmers to a new low-cost, food bait hornet trap for efficiently reducing the menace of hornets in the region.

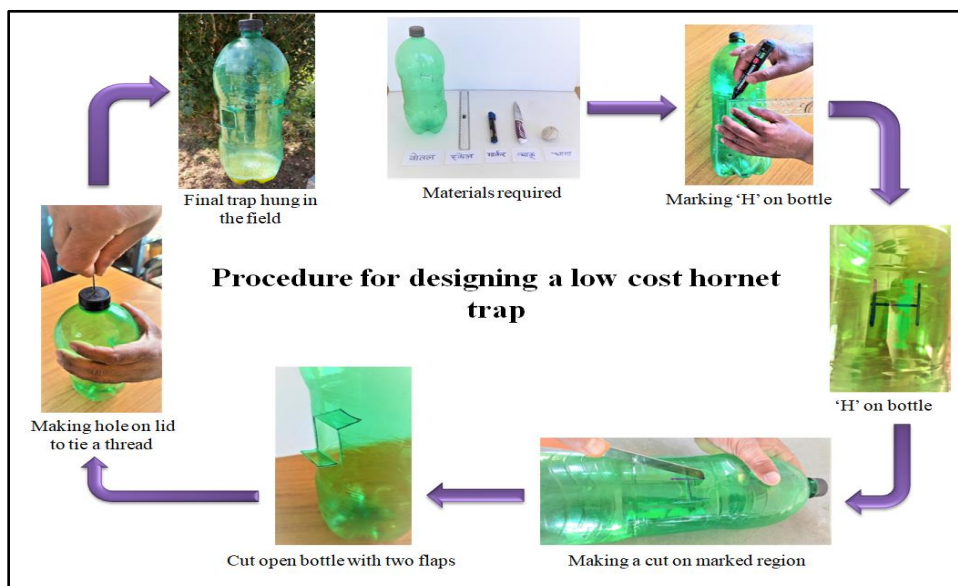
## Methodology

### **Identification, biology, predatory and nesting behavior of native hornets**

One representative specimen of *V. mandarina*, *Vespa velutina nigrithorax* and *Vespa tropica* were caught using a hand net and morphologically characterized through the literatures of Mason and Huber, [15] and Seltsmann, [16] by pictorial, interactive and dichotomous keys. The biology of a colony was studied in detail by year round monitoring of the hornet colony and predatory behavior was examined by observing its predation and feeding habit during the predation cycle in May to November 2020 and 2021. The nesting behavior was studied by cutting open the large nests constructed in rodent burrows and tall trees at Experimental Farm, Hawalbagh, ICAR- Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand, India (29°38'01" N and 79°37'49" E, altitude 1250 AMSL) which comes under the Alpine and Humid subtropical climatic zone of India.

### ***Designing of the bait trap***

To design a low-cost hornet trap, locally available bait materials were used. Wherein, a green-colored and transparent plastic bottles were washed thoroughly and marked on the three sides with a 2.5 cm 'H' shaped design (Figure 1). The bottles were cut on the 'H' mark with a sharp knife to create two flaps. The upper flap was folded to the inside and the lower flap was folded to the outside (the lower flap acts as a platform for hornets to rest and enter the bottle). A small hole was made on the lid to tie a thread for hanging the bottles.



**Figure 1. (A) Procedure for designing of a low cost hornet trap**

### ***Bait materials and their optimum proportions***

The three main bait ingredients (apple cedar vinegar, orange juice, and water) (Table 1 and Figure 2) were used along with supplementary materials like one tablespoon of market honey, detergent powder (2%), and rotten fruits (5 grams) to formulate the bait material. A total of five test proportions (1:1:3, 1:3:1, 2:2:1, 1:2:2, 2:1:2) (wherein, 1:1:1 equals 25 mL: 25 mL: 25 mL) of three major ingredients (Apple cedar Vinegar: orange juice: water) were evaluated for their efficacy in attracting and trapping the hornets (Figure 3) and the experiment was replicated four times to reduce the anomalies.

**Table 1. List of ingredients required to design one low cost hornet trap**

SN.	Ingredient	Quantity per trap	Proportion to be added	Cost per trap (in INR)
1.	Green plastic bottle	1	-	-
2.	Apple cedar Vinegar/ wine	25 ml	1	12
3.	Orange juice	50 ml	2	5
4.	Water	50 ml	2	-
5.	Detergent powder	1 gram	-	≈ 1
6.	Rotten banana	1-2 pieces	-	-
7.	Market honey	1 table spoon	-	≈ 2
			<b>Grand total</b>	<b>≈ 20</b>

Note: ≈ indicates the approximate cost

Additionally, two types of bottles, green colored and transparent bottles were evaluated for their trapping efficiency of three hornet species (*V. mandarinia*, *V. velutina nigrithorax* and *V. tropica*) and the non-target honey bees (Figure 3). The number of insects of different species trapped was recorded on daily basis and the bait solution was reused up to 8-10 days without loss of efficiency.

**Benefit cost ratio of hornet trap vs. traditional method**

A total of ten newly designed hornet traps were installed in an apiary with 25 bee hives at Experimental Farm, Hawalbagh, Almora, Uttarakhand, India (29°38'01" N and 79°37'49" E, altitude 1250 AMSL) and the traditional method of hornet management was practiced at farmers' fields at Mehatgaon, Almora, Uttarakhand, India (29°65'32" N and 79°62'96" E, altitude 1280 AMSL) with 25 bee hives. The benefit-cost ratio of the two methods was compared in monetary terms with respect to honey yield obtained in both the apiaries (details in Table 2).



**Figure 2. Procedure for preparation of bait solution for trapping hornets**

**Table 2. Comparison between benefit cost ratios of trap vs. traditional method**

Materials and labour	Cost in traditional method (in Rs)	Cost for installation of the trap (in Rs)
Cost of ingredients	Nil	20 per trap
Cost of labour	267 per day	Approx Rs. 267 for designing 10 traps
Cost of labour per month	8000 per month	10 traps per apiary X changed 4 times a month= 800 Rs
Total cost per month	8,000 per month	1067 per month
Total cost for 3 months during peak infestation	24,000	3,201
Total honey produced per apiary (average)*	87.5 kg X 450**= 39,375	= 39,375
<b>B:C ratio</b>	<b>1.64:1</b>	<b>12.30:1</b>

**Note:** \*an average apiary in the Indian Himalayas consists of 25 bee hives with average honey yield of 3.5kg per hive per year, established in an area of 200 m<sup>2</sup>. So, 10 traps are sufficient to cover an area of 200 m<sup>2</sup>  
\*\*average cost of honey in the Indian Himalayas is 450 Rs/ Kg



### **Statistical analysis**

The experiments were set up in a completely randomized block design (CRBD) with five test proportions of three major ingredients and replicated four times. The trapping efficiency of bait ingredients was analyzed by calculating the average values of different hornet species trapped through Microsoft Office Excel 2019 (Microsoft corp., USA) and the ANOVA was assessed at  $p < 0.05$  level of significance. The F-values, SE (m), and CV (coefficient of variation) values were calculated through SPSS software to compare the means. Additionally, the Principal Component Analysis (PCA) was performed to ascertain the effect of supplementary bait ingredients on the trapping efficiency of traps towards different hornet species.

## **Results and Discussion**

### **Important hornet species: identification, biology and predatory behavior**

#### **1. Giant Asian hornet (*Vespa mandarinia*)**

It is the largest hornet in the world and is known to have originated in temperate and tropical East Asia and South Asia [17]. They prefer to live in low mountains and forests, while almost completely avoiding plains and high-altitude climates. They are social hornets that create nests by digging the ground or adapting to pre-existing tunnels of rodents or occupying spaces near rotted pine roots in the mid and low hills of the Himalayas [18].

#### **Identification**

*V. mandarinia* is a large hornet with a body length of 45 millimeters and wingspan of 70 mm. The sting is around 6 mm long and can inflict a very painful sting and inject a large amount of potent venom. The hornet's head is yellowish-orange in color with a brown antenna. The eyes and ocelli are dark brown to black with large clypeus and gena. The mandibles are dark brownish with a black distal portion, very strong, and used for digging the ground and decapitating the prey. The thorax and abdomen are dark brown or black with a yellowish-orange hue. The sixth abdominal segment has a sternal gland, also known as van der Vecht's gland. The scent produced from these glands is used to mark the food source. During September and October, the hornets mark the bee colony to attack in groups for mass slaughtering of the bees [18-20].

#### **Biology and life cycle**

The life cycle of *V. mandarinia* consists of six phases (Pre-nesting phase, solitary phase, cooperative phase, polymathic phase, dissolution phase, and hibernating phase) and it is consistent with other eusocial hornets. Both the uninseminated and inseminated females end the hibernation period and emerge in mid-April months. The uninseminated females start feeding on the sap of oak trees (*Quercus* spp.), while, the inseminated females are initially solitary and along with other females initiate the construction of subterranean nests. By the end of July, a fully developed nest with 500 cells and 100 workers is formed and the queen restricts itself to the colony and worker hornets start foraging. This cooperative phase continues up to late September and once the winter commences (early October), the queen hornet stops laying eggs and shifts its focus to caring for the young ones. The queen dies in late October and the responsibility to produce a new queen is taken over by drones and workers. The drones and workers mate mid-air and form inseminated females, while the unmated females are left as uninseminated females. Once again both the females undergo hibernation to start a new colony cycle in the next season [21-22].

#### **Predatory behavior**

The Asian giant hornets primarily feed on larger insects, other eusocial insects, tree sap, and honey bees. However, honey bees are easy targets. The worker hornets wait at the hive entrance, capture the foraging bees, cut apart their head and abdomen and carry the nutritious thoracic region of



the bees to their nest for mass provisioning of their broods. Usually, one hornet can hunt up to 40-60 bees in its hunting phase and 120-150 bees in its slaughtering phase. If the bee colony is weak, the hornets enter the hive, collect grubs, pupa and honey from the hive and abandon the empty and weak colony.

## **2. Yellow-legged hornet (*Vespa velutina nigrithorax*)**

The hornet is native to north-eastern India, and southern and central China [23]. *V. velutina* occurs in tropical regions, but the nests are normally found in the cooler highland or upland regions [24]. The hornet can adapt to new environments, urban and sub-urban areas, agricultural lands, forests, and wooded areas [25].

### **Identification**

The size of this hornet is much smaller than *V. mandarinia* and can easily be identified by its hovering nature. The body size is approximately 23-26 mm with a wingspan of 32-44 mm. The hornet has a dark brown body with only one orange-colored band on the 4<sup>th</sup> tergite of the abdomen. The tarsal leg segments are yellowish and the remaining segments are dark brown to black [18, 20].

### **Biology and life cycle**

Like other hornets, they too follow an annual life cycle. The hibernating mated females emerge in early spring and construct embryo nests (the size of a tennis ball) with 50-100 workers and rear their first brood. For the second brood, the colony is relocated to tall trees or buildings where the colony expands with thousands of workers and a single queen restricted to egg-laying in the colony. During this phase, hornets hunt insects and provision their broods with nutritious food. In the winter season, the colony produces thousands of sexuals (males and new queens) that mate outside the colony. The drones starve to death, while the mated and unmated females hibernate to start a new colony cycle in the next year [14].

### **Predatory behavior**

It is a honey bee predator that mostly prefers the European bee (*Apis mellifera*) over the Indian bee (*Apis cerena indica*) because the Indian bees have co-evolved with their hornet pests and have developed two advanced defense mechanisms like performing Mexican waves in unison by shimmering their wings and enclosing the attacking hornet in a ball of vibrating bees to create a high temperature (450C) and kill the hornet [26-27]. These two defense mechanisms are absent in *A. mellifera* and thus these colonies are highly susceptible and exposed to the attacks of *V. velutina*. These hornets can hunt up to 30 bees in a day and carry them to the nest for mass provisioning to their broods, while, the slaughtering phase can be observed in weak bee colonies leads to deserting and abandoning of the colony by bees [28].

## **3. Greater banded hornet (*Vespa tropica*)**

It is a tropical species of hornet known to be distributed in Southern Asia, West Africa, and New Guinea [29]. But, recently it has invaded different parts of the Pacific islands. It mainly predares on paper hornets, larvae in the field, and rarely on honey bees. It has a very well-developed sting that can cause extreme pain and swell in humans [30].

### **Identification**

It is a large black to brown colored hornet with a reddish to brownish head and with a distinct yellow-colored stripe on the second abdominal segment. However, variation in the abdominal coloration is observed in various geographical locations. The size of a worker hornet ranges between 24-28 mm in length and wingspan of 34-36 mm. It is often confused with *Vespa affinis* which has yellow stripes on both 1<sup>st</sup> and 2<sup>nd</sup> abdominal segments. *V. tropica* is mainly associated with forests



and lowlands up to 2100 m altitude [31].

### ***Biology and life cycle***

The life cycle of *V. tropica* resembles that of *V. mandarinia* and *V. velutina* nigrithorax. However, the nesting behavior is very peculiar, wherein, the hornets construct their nests in tree hollows 3 m above the ground with open bottom. Moreover, the nest is quite brittle, consists of broad and distinct outer layers and the population of each colony fluctuates around 100-250 individuals. The nesting by *V. tropica* is also recorded in dead logs, building roofs, sheds, and tree crowns in rare cases [32-33].

### ***Predatory behavior***

The adults of *V. tropica* usually predate on larvae infecting the crops, dragonflies, and other large insects. They sometimes capture the foraging bees from the fields and carry them back to their nest for provisioning the food to their offspring. They rarely attack the bees near the hive and the behavior of waiting on the bee board and bee entrance is usually not observed in the case of *V. tropica*. They are very serious pests on foraging bees in Indian Himalayas in monsoon months when bees are actively foraging [34-35].

### ***Evaluating the optimum proportions of bait materials***

To evaluate the efficacy of newly designed hornet traps, an apiary was established at Experimental farm, Hawalbagh, ICAR-VPKAS, Almora (29.63°N and 79.63°E, 1250 m) was chosen. The open forest area, presence of wasp nests in nearby forests, and regular, the severe incidence of three hornet pests in the apiary made it a suitable site for conducting the experiment. The months of August, September, and October were selected for trap installation, as the pest incidence was very severe during these months and up to 15 hornets were seen attacking a single bee colony and more than 120 bees were decapitated per day. The period of hornet infestation on the bee colonies in the study area coincided with the studies of [34-36]. A total of five test proportions or ratios (1:1:3, 1:3:1, 2:2:1, 1:2:2, 2:1:2) of three major ingredients (Apple cedar Vinegar: orange juice: water) were evaluated for their efficacy in attracting and trapping the hornets (Figure 3). Two types of bottles, green-colored and transparent bottles were evaluated for their trapping efficiency. The observations for three hornet species (*V. mandarinia*, *V. velutina nigrithorax* and *V. tropica*) and honey bees trapped were recorded on daily basis and the bait solution was reused for up to 8-10 days without loss of efficiency. It was observed that, among the two bottles used, the transparent bottles were less efficient in trapping the hornets; while, green bottles were significantly superior in performance with the highest number of hornets trapped in ratios 3, 4, and 5 (Figure 3). Moreover, the Coefficient of variation of green bottles was far lower (22.49, F-value= 13.46) than the transparent bottles (43.37, F-value= 29.87), thus indicating the consistency in trapping of hornets by green bottles. Among the three ratios, ratio 4 (1:2:2) performed significantly better than others (as compared by SE(m) values) and the cost for the preparation of ingredients was also much lower (20 Rs per trap). Moreover, the ratio 4, trapped the highest number of *V. velutina nigrithorax* and *V. tropica*, while, ratio 5 trapped the highest number of *V. mandarinia*. Although ratio 3, performed equally well with ratios 4 and 5, it was not significantly superior to ratio 4 and the cost of preparation was also high (34 Rs per trap). However, ratios 1 and 2 showed significantly poor trap catches of the target hornets and the possible reason could be the lesser quantity of attractants like apple cedar vinegar and orange juice or excessive dilution of the bait ingredients. The further fine-tuning of trap ingredients were necessary to reduce the trapping of honey bees and increase the hornet trapping efficiency of traps. Similar studies were conducted by Glaiim et al., [37], who reported that vinegar-based traps were inefficient in trapping the oriental hornet (*Vespa orientalis* L.), attacking the honey bee, *Apis mellifera* L. in Iraq. Whereas, Belkavsky [38] showed that, the scent of vinegar attracts wasps and hornets in large numbers and can be used for trapping them in apiaries in Italy.

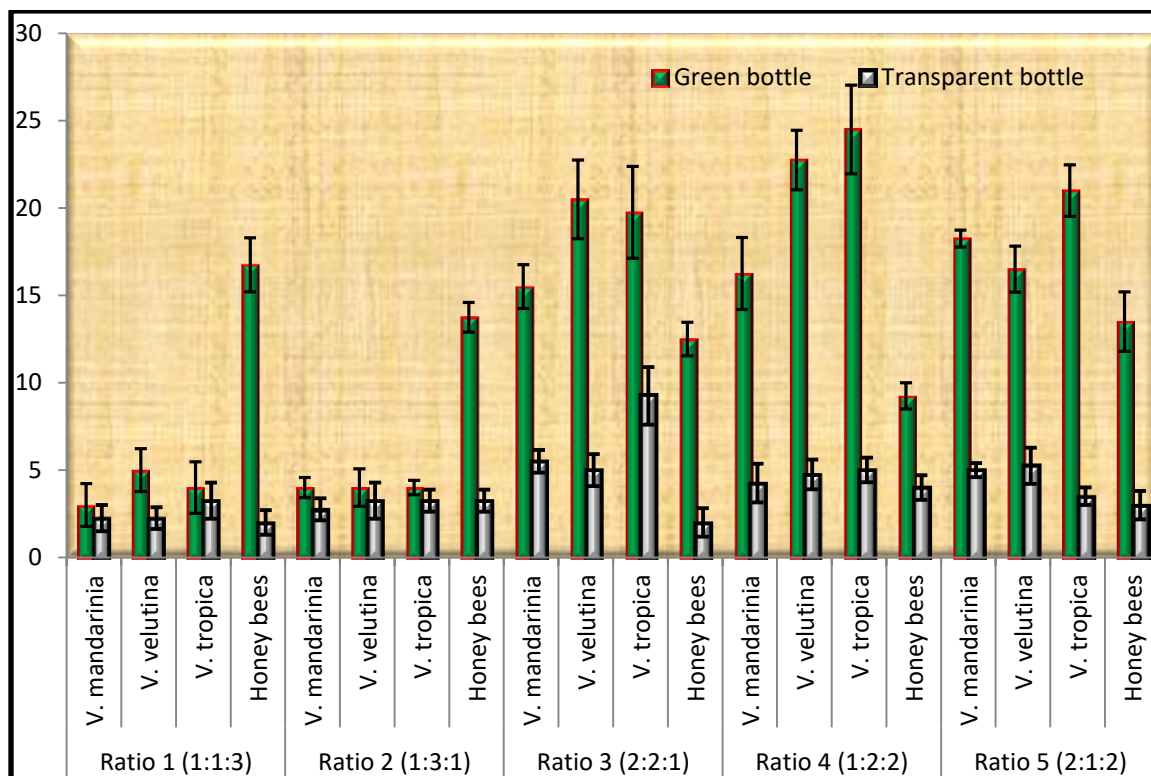


Figure 3. Testing the trapping efficiency of different proportions of bait ingredients (Apple cedar Vinegar: orange juice: water)

### Importance of market grade honey and rotten fruits in enhancing the trap efficiency

The addition of two supplementary bait ingredients; honey (market grade) one teaspoon and two pieces of rotten fruits (5 grams) played an important role in improving the efficiency of the trap, by repelling the honey bees and attracting only hornets. The PCA analysis was performed to ascertain the effect of supplementary bait ingredients on the trapping efficiency of traps for different hornet species. The effect of the addition of fruits and honey is presented in Fig 4a and 4b. The principal component 1 and 2 accounted for ~85 and 76% of total variance with fruit and honey-based substrate, respectively. An angle of zero or 180° reflects a correlation of 1 or -1, respectively. The superimposition of various treatments to test the efficiency of attracting and trapping hornet species showed that the inclusion of rotten fruits into bait ingredients exhibited a positive correlation for *V. mandarinia*, *V. velutina nigrithorax* and *V. tropica* while honeybees remain attracted more towards the fresh fruits (Figure 4a). Similarly, *V. mandarinia* and *V. tropica* trapping increased with the addition of market honey to bait ingredients while *V. velutina nigrithorax* preferred bait solution without honey (Figure 4b). However, the addition of hive honey from the apiary resulted in attracting and trapping more honey bees into the trap, which may be because of the presence of colony odor in the local bee hive honey. Therefore, the addition of supplementary bait ingredients like rotten fruits and market honey improved the trap efficiency by enhancing attractiveness to hornets and avoiding the trapping of honey bees. Similar studies were conducted by Bacandritsos et al., [39], who used animal-based food baits (fish and meat) for attracting and trapping the two hornet species *Vespa orientalis* L. and *Vespula germanica* (F.). They concluded that the animal-based baits were effective only for two days and they trapped the hornets effectively only during the morning hours of the day.



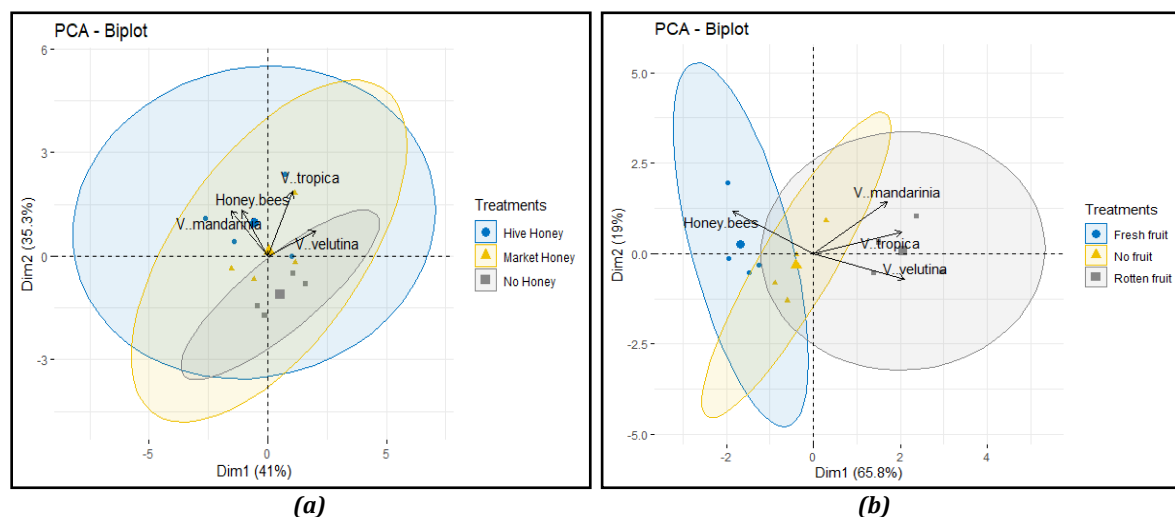


Figure 4 (a) and (b). PCA analysis to ascertain the effect of supplementary bait ingredients on trapping efficiency of the hornet trap

### Traditional method v/s low cost hornet trap

Although Indian bees have co-evolved and developed several defense mechanisms, they cannot completely avoid the damage/predation by hornets [26, 35]. However, the European bees are highly susceptible to hornet attack and they lack both the defense mechanisms of shimmering wings to create Mexican waves and making heat balls to kill the hornet [26-27]. The traditional management practices like swatting predating hornets near the bee hives, locating the nests in late winter months to kill the hibernating queen and workers, and killing of actively laying queen in the monsoon months are followed by farmers in the Indian Himalayas [6]. Moreover, few farmers practice fitting entrance reducers to the hive to prevent the entry of hornets into the hive but this does not reduce the predation of forager bees. Martin [13] also recommended the use of specific strains of parasitic nematodes, Entomopathogenic fungi, and poison baits for managing the menace of hornets in the apiaries but, these tactics have rarely been successful [14]. The infestation of hornets in the apiary in Indian Himalayas usually begins from August and continues up to the end of October. Farmers have to employ a labor for 3 months to manage the hornets in a traditional method of pest management. Considering the severity of attack and the amount of loss incurred to the apiarists of Indian Himalayas, a low-cost bait trap was designed and evaluated at ICAR- Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand, India. The low-cost trap can be easily designed by farmers with locally available materials. The enhanced specificity of the trap against hornets makes it the most suitable device in the apiary for hornet management. The cost incurred on labor for manually swatting the hornets is reduced by more than 80% and health hazards from hornet stings are also reduced drastically. Moreover, the installation of a trap can reduce the cost incurred on the labor and in turn protect humans from hornet stings. The benefit-cost ratios of both traditional management methods and trap installation are furnished in detail in Table 2. It can be observed from the study that, using a low-cost hornet trap could yield a benefit-cost ratio of 12.30:1, when compared to the traditional method of manual swatting of hornets yielding a B:C ratio of 1.64:1, which is significantly lower.

### Conclusion and future prospects

The hornets are emerging as one of the very serious pests of honey bees in Indian Himalayas in recent years and farmers are facing a huge economic crisis in terms of direct colony loss, reduction in honey yield, and high investment in labor for manual swatting of the hornets. However, the development of low-cost, simple design trap by the scientists of ICAR-VPKAS, Almora may help the



farmers to reduce the menace of the hornets and improve the economic yield of honey. Moreover, further modifications in the trap design, refining of the bait materials, and making the bait more attractive to the hornets can pave a path for a future line of work. The development of synthetic pheromones to attract hornets is also under study and needs further investigation. Although a large number of hornets preying on bees near the hive or apiary are trapped in the trap, the predation of forager bees from the fields is difficult to manage and there is an immediate need to develop efficient, large distance pheromone to attract and kill the preying hornets. An important point to consider is that, even though these hornets are notorious pests of honey bees, they also predate on caterpillars and other insect pests and play a role in natural pest management in the agro-ecosystem. So, considering their importance, suitable tactics have to be designed to manage the hornet damage in the apiary without disturbing the biodiversity in the natural ecosystem.

### Conflict of Interest

All the authors have thoroughly reviewed the research article and declare no conflict of interest

### Authors' contribution

AUP- Designed the hornet trap, collected the data, and drafted the manuscript, ARNSS- Conducted studies on different bait proportions and collected data, MP- compiled and analyzed the data, SB- Final edition of the manuscript, KKM- language editing, LK- Final revision of the manuscript. All authors read and approved the final manuscript.

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