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Chapter 11

Integrating Sniffer Bees into Security and Defenses: Advancements in the Detection of Explosives and Illicit Substances

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Abstract

Warfare and insects have always maintained a hidden persistent relationship, with insects being weaponized throughout history for entomological warfare. Several techniques have been used to utilize honeybees as weapons, including bee catapults, bee manikins and bee grenades, aimed at national defense. The advent of landmines in the 19th century introduced a potent, cost-effective means of defense against adversaries. Among methods for landmine detection, biological approaches involving honeybees and wasps have emerged, exploiting their innate behaviours. Techniques such as the Free Flight Method, Proboscis Extension Reflex and Hybrid Insect Micro-Electro-Mechanical Systems manipulate honeybee behavior to maximize their efficiency in detecting landmines. Pavlovian classical conditioning underlies this training, where honeybees are exposed to explosive odours followed by immediate sugar syrup rewards. Tracking the spatial pattern of honeybees using LIDAR technology enables the identification of suspected landmine sites. Technological advancements like the VASOR-136 training box developed by Inscentinel Ltd. enable the training of up to 36 bees simultaneously. Pre-concentrator technology, analyzing Aflas-coated sheets at hive entrances, is another effective method for landmine detection. The sensory mechanisms of bees, including antennal sillia, antennal lobe, mushroom body and lateral horn, harbour olfactory receptor neurons crucial for olfaction. Beyond landmine detection, advancements in sniffing technology allow these bees to aid in the early diagnosis of diseases like cancer and diabetes, environmental monitoring, search and rescue missions, security, law enforcement, urban planning and pest management. Ongoing research continues to elucidate the intricacies of their sensory mechanisms, positioning sniffer bees to play an increasingly pivotal role in fostering a sustainable, secure and sustainable future for both humanity and the ecosystem.

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1. Introduction

The intersection between warfare and insects has remained quietly influential, primarily through the role of vectors in disease transmission. Lice, ticks and fleas have historically spread typhus and plague, while mosquitoes have facilitated the spread of malaria and yellow fever. War conditions often involve overcrowding, population displacement, inadequate healthcare, poor sanitation and widespread malnutrition; all of which increase vulnerability to disease and create conditions ripe for epidemics (Peterson, 1995). Insects have been exploited as biological weapons not only through natural disease transmission but also in three strategic forms: dissemination of pathogens to enemy forces, release of pests to damage agriculture and direct attacks on humans to cause physical or psychological distress (Lockwood, 2008).

Honeybees represent an important group of hymenopteran insects mainly as economically important insects due to their social behaviour and useful products. Historically, honeybees have served in both offensive and defensive roles. A modern defensive use under development is their deployment in landmine detection. Since the mid-19th century, landmines have been cheap, easy to produce and highly effective weapons of war (Delaney, 2011). Landmines serve three key roles in warfare: disrupting enemy coordination, inflicting damage on personnel and vehicles and instilling fear among opposing forces (Lapointe, 2009). Honeybees are highly efficient foragers, covering vast areas and visiting nearly 5 million flowers to produce a single pint of honey. Their bodies are equipped with branched hairs that generate a static charge, enabling them to collect environmental particles. Upon returning to the hive, researchers can analyze these particles, along with honey and beeswax-to monitor environmental contaminants.

The concept behind training honeybees to detect explosives is “Pavlovian conditioning” where the honeybees are exposed to odorant molecules with immediate sugar syrup reward (Rehman *et al.*, 2022). Honeybees are also potentially proven to detect some of the illicit substances which are mostly exploited by drug trafficking *viz.*, heroin, Ganja, opium, *etc.* (Schott *et al.*, 2015). This paper entails the use of sniffer bees to detect landmine explosives as well as other illicit substances. Apart from these illicit substances uses of sniffer technology in the detection of disastrous diseases like cancer, tuberculosis and diabetes are also described in detail.

2. Conventional Use of Honeybees in War

2.1. Bee Catapult

In ancient warfare, Romans didn’t just use swords they hurled chaos. Beehives, flung over garrison walls or straight into enemy ranks, turned buzzing insects into weapons. The practice was so intense; it's believed the Romans nearly ran out of bees (Delaney, 2011).

2.2. Bee Manikin

The Popol Vuh, a sacred Mesoamerican manuscript, recounts how ancient Mayans in Guatemala used decoy warrior figures to create the illusion of a larger army. These manikins had hollowed gourd heads filled with bees, which, when broken during enemy attacks, unleashed chaos and gave the Mayans a strategic advantage (Lockwood, 2008).

2.3. Bee Grenade

The Mayans devised an ingenious method of turning bees into weapons by crafting hollow clay balls with small entry holes. These were placed near beehives, encouraging bees to move in and nest. Before going into battle, they sealed the holes with grass, enough to confine the bees but still allow airflow. During combat, these "bee bombs" were thrown into enemy lines, where they shattered and unleashed a swarm of enraged bees, creating confusion and panic (Lockwood, 2008).

3. Disputed Borders of India

India has several disputed regions, each rooted in intricate historical, geopolitical and cultural factors. Among the most notable is Kashmir, which has been a longstanding point of contention between India and Pakistan, leading to numerous conflicts and ongoing tensions. Another disputed area is the region of Arunachal Pradesh, claimed by both India and China, with border disputes occasionally escalating into military confrontations. Additionally, there are disputes over territories such as the Sir Creek area and parts of the India-China border in areas like Aksai Chin. These disputes play a critical role in shaping the security dynamics of the regional stability and international relations, highlighting the intricate challenges India faces in managing its territorial integrity and diplomatic relations.

4. Different Methods of Landmine Detection

4.1. Biological Detection

Biological sensors or biosensors such as dogs, some rodents, honeybees, some types of plants and some types of bacteria depend on the possibility of direct sensing of explosive compounds (Habit, 2007). All the organisms are trained properly by using the Pavlovian conditioning method for effective detection (Kasban *et al.*, 2010).

4.2. Electromagnetic Detection

Landmine detection *via* electromagnetic radiation relies on disparities in electromagnetic characteristics between the target and the ground (Sen and Woodfin, 2002). Various electromagnetic techniques are utilized, differing in frequency, bandwidth, signal type, signal interpretation and equipment type. Common methods include metal detector (MD), ground penetrating radar (GPR), microwave radar (MWR), millimeter wave radar (MMWR), electrical impedance tomography (EIT) and infrared (IR) techniques.

4.3. Metal Detector Technique

This technique employs electromagnetic induction (EMI) with primary and secondary coils (Masunaga and Nonami, 2007). Time-varying current in the transmitter coil generates an electromagnetic field inducing eddy currents in metal objects. Detects <1 cm metals at 50 cm depth, cost-effective and reliable in all conditions.

4.4. Ground Penetrating Radar (GPR)

GPR systems emit electromagnetic pulses into the ground and analyze the reflected signals to identify changes in material density, indicating buried objects such as landmines (Mans, 2006). GPR technology is employed in demining operations to survey large areas quickly and accurately, mapping out subsurface anomalies that may represent buried landmines.

4.5. Thermal Imaging

Thermal imaging cameras detect temperature variations on the ground's surface caused by differences in thermal conductivity between landmines and surrounding soil. Infrared sensors mounted on unmanned aerial vehicles (UAVs) or handheld devices capture thermal images of the terrain, enabling the detection of buried landmines based through their distinct thermal signatures.

4.6. LIDAR (Light Detection and Ranging)

Light Detection and Ranging technology presents a viable approach to landmines detection by providing high-resolution terrain mapping and identifying subsurface anomalies. Operating by emitting laser pulses and measuring the time it takes for the light to return after reflecting off surfaces, LIDAR generates detailed three-dimensional maps of the ground (MacDonald *et al.*, 2003). In landmine detection, LIDAR can detect disturbances in the soil caused by buried objects, including landmines, based on variations in surface elevation. By analyzing these elevation changes, LIDAR systems can help demining teams identify potential landmine locations more efficiently and accurately than traditional methods. Additionally, LIDAR can be mounted on drones or ground vehicles, allowing for rapid and remote surveying of mine-affected areas without putting personnel at risk. Overall, LIDAR technology offers significant potential for enhancing the effectiveness and safety of landmine detection and clearance efforts.

5. Training Process in Honeybees

There are three different processes in the training honey bee:

i) Free Flight Method

ii) Contained Detection Devices/ Proboscis Extension Reflex (PER)

iii) Hybrid Insect Micro-Electro-Mechanical Systems (HI-MEMS)

5.1. Free Flight Method

Scientists have developed a method to train honeybees to recognize specific explosive-related scents by adding tiny amounts of landmine by-products to their food. The bees quickly form an association between the chemical odor and a food reward. To boost their learning and memory, caffeine is included in the training process. After just a day or two of exposure, an entire colony, around 40,000 bees, can be deployed in minefields, where they instinctively track the scent of explosives, ignoring flowers and plants. This training approach is low-cost, requires minimal labor and makes it possible to condition large numbers of bees simultaneously to detect various types of explosive materials. Trained bee will be attracted towards the odour of explosives and they will be accumulated near the suspected place of landmine buried. The LIDAR technique will be used to detect their location by which approximate suspected location sites will be identified.

5.2. Contained Detection Devices/ Proboscis Extension Reflex (PER)

Los Alamos National Laboratory (LANL), with DARPA backing, developed a novel bio-sensing method through the Stealthy Insect Sensor Project. By exploiting the Proboscis Extension Reflex (PER), a natural response in bees to food-related odors-researchers trained bees to react similarly to explosive compounds. These responses are captured in mobile detection units, where behavioral data is processed through pattern-recognition software and displayed in real time on PDAs. The technology has successfully detected explosives across various contexts, demonstrating high accuracy and adaptability.

5.2.1. VASOR-136

Researchers at Los Alamos National Laboratory (LANL) created a prototype explosive-detection device known as the “sniffer box”, a shoebox-sized unit containing three restrained bees. A camera inside the box focused on the bees’ heads and relayed footage to a laptop. When the device was activated, air samples were drawn from the surroundings and passed over the bees. The bees remained unresponsive to clean air but exhibited the Proboscis Extension Reflex (PER) when exposed to air carrying trace levels of explosive vapors. Building on this concept, UK-based biotechnology firm Inscentinel Ltd. developed an advanced version of the technology, the VASOR-136 detection unit. Unlike LANL’s prototype, which required a human operator to interpret bee behavior *via* live video feed, the VASOR-136 eliminates the need for manual observation. It utilizes Volatile Analysis by Specific Olfactory Recognition (VASOR), integrating a camera with motion and pattern-recognition software to analyse bee responses. These behavioral patterns are then converted into electronic signals and displayed on a Personal Digital Assistant (PDA) screen. The VASOR-136 is a compact, handheld device resembling a car vacuum. To operate it, trained bees are first cooled (a harmless process that slows their movement), then individually secured in humane, reusable harnesses. A total of 36 bees are distributed into six cassettes, which are inserted into the device after a brief acclimatization period.

5.3. Hybrid Insect Micro-Electro-Mechanical Systems (HI-MEMS)

Since 2007, DARPA has pursued a pioneering defense initiative known as the Hybrid Insect Micro-Electro-Mechanical Systems (HI-MEMS) project, which aims to engineer cybernetic insects, essentially creating biological-cybernetic hybrids using bees and other species (Delaney, 2011). This technique involves embedding microelectronics into insects like honeybees, could revolutionize landmine detection efforts. By leveraging the remarkable olfactory capabilities and innate foraging instincts of bees, these tiny bio-drones can be trained to detect specific chemical signatures associated with landmines. Equipped with miniaturized sensors and wireless communication devices, the bees would relay information regarding the detection of landmines n real time to a central control system. This cutting-edge strategy provides several advantages, including the capacity to cover large areas quickly and access hard-to-reach or hazardous terrain (Bozkurt *et al.*, 2008). Additionally, using bees for landmine detection is environmentally friendly and reduces the risk to human deminers. Overall, HI-MEMS technology has the potential to significantly enhance the efficiency, safety and scalability of landmine detection efforts. This is a type of Cyborg technology and it was developed by Defence Advanced Research Project Agency (DARPA) of the USA. It was initiated in 2007 in four locations *viz.*:

i) University of Michigan (Flower beetle and honeybee)

ii) University of California at Berkeley (June beetle and honeybee)

iii) Boyce Thompson Institute at Cornell University (Tobacco hawk moth)

iv) Massachusetts Institute of Technology (Honeybee)

5.3.1. Applications of HI-MEMES

Field of Application	**Uses**
Search & rescue	• Roaches are sent to disaster zones to seek out humans trapped under rubble. • Used to save the lives of disaster victims.
Military context: Information gathering	• Cyborg insects can sniff out bombs. • Example: Cyborg locusts are developed for chemical sensing (Mehta et al., 2017).
Military context: Information gathering	• Remote-controlled cyborg beetles could spy on terrorists.

6. Pre-Concentration Technique

6.1. Examination of Aflas-Coated Surface

The preconcentration technique for landmine detection utilizing honeybees involves a sophisticated method leveraging the natural behavior of bees to detect and collect trace amounts of explosives emitted from landmines. In this method, preconcentrators are strategically positioned at the entrances of beehives, where bees naturally congregate. These preconcentrators typically consist of specialized substrates coated with a polymer material such as Aflas®, recognized for its strong binding capacity to explosive compounds. The substrate, often Whatman filter paper strips, is blade-coated with Aflas solution, forming a surface capable of adsorbing and retaining explosive molecules. These coated strips are rolled up to make tubes and inserted into plastic cartridges, which are placed at hive entrances. As the bees enter and exit the hive, they unintentionally encounter with these preconcentrators, transferring minute amounts of explosive particles onto the Aflas-coated surface (Gillanders *et al.*, 2019). Over time, as bees continue their routine activities, more particles accumulate on the preconcentrator, increasing the concentration of explosives captured. After a specified collection period, typically ranging from one to seven days, the preconcentrators are carefully removed from the hive, sealed and stored for subsequent analysis. The acquired samples undergo further examination *via* laboratory testing to identify and quantify the presence of explosives. This innovative technique offers a non-invasive and efficient means of detecting landmines, capitalizing on the natural foraging behavior of bees to provide a sensitive and reliable method for locating explosive hazards in the field.

6.2. Examination of Air Drawn from Honeybee Hive

When the air from within the honeybee hives is sampled using vacuum pumps attached to specialized nozzles, the preconcentrators capture and retain a larger volume of air containing concentrated explosive particles. This enriched air sample, obtained from the hive environment, contains a higher density of target molecules compared to ambient air, increasing the likelihood of detecting even extremely low concentrations of explosives. By drawing air directly from the hive, the preconcentration technique maximizes the efficiency of collecting airborne particles associated with landmines, thereby improving the sensitivity and accuracy of the detection process. Furthermore, the use of preconcentration allows for the selective enrichment of explosive compounds, reducing interference from background contaminants and improving the signal-to-noise ratio during subsequent analysis. This selective concentration enhances the detection capabilities of analytical instruments, such as gas chromatography-mass spectrometry (GC-MS) or ion mobility spectrometry (IMS), enabling more reliable and precise identification of explosive residues. Overall, by harnessing the foraging behavior of honeybees and employing preconcentration techniques, the detection of landmines from air drawn from honeybee hives becomes more effective, sensitive and practical, offering a promising approach for enhancing the efficiency of landmine detection and clearance efforts in various environments.

7. The Sniffer Wasp: Food Contaminant Detector

Microplitis croceipes has been extensively studied for its ability to effectively search for hosts and nectar sources. Researchers have conducted several studies to explore the wasp's capacity to learn, remember, distinguish and react to different smells, both in its natural environment and under controlled conditions (Takasu and Lewis, 1996; Rains *et al.*, 2009). In experiments using the Cyranose 320 electronic nose (Smiths Detection, Inc.), *M. croceipes* was found to be highly sensitive to chemicals like myrcene and 3-octanone, being nearly 100 times more sensitive compared to other substances (Rains *et al.*, 2004). When exposed to target volatile chemicals, these parasitic wasps exhibited positive responses, characterized by movements of their bodies and antennae towards the source of the odor, particularly when the unconditioned stimulus was food. Moreover, research findings suggest that the behavioral responses of *M. croceipes* vary based on the context associated with the odor, such as the type of resource it was linked to (Olson *et al.*, 2003). For instance, in experiments, the wasps were conditioned to respond to one odorant while feeding on sugar water. Later, after at least 15 minutes, the same wasps were conditioned to respond to a different odorant while stinging their host, *Helicoverpa zea*.

As a result, each *M. croceipes* wasp exhibited either foraging behavior (in response to food odors) or stinging behavior (in response to host odors) when presented with the respective conditioned odorants. Additional research has shown that these wasps can also distinguish between odors that are chemically similar (Meiners *et al.*, 2002). Future studies are necessary to explore how combinations of odors affect recognition by the olfactory system. To harness the abilities of *M. croceipes* as a sensor, a device known as the Wasp Hound was developed. This device has been utilized to detect odors associated with Aflatoxin in food products (Rains *et al.*, 2006) and animal carcasses (Tomberlin *et al.*, 2008). The Wasp Hound works by housing the wasps in a small cartridge through which air samples are passed. A web camera captures the wasps' behavior and the Wasp Hound, connected to a software program called Visual Cortex, analyzes these responses in real-time, presenting them as graphical data (Utley *et al.*, 2007).

7.1. Phases of Training of Sniffer Wasp

7.1.1. Identification of Target Odors

Researchers identify the specific volatile organic compounds (VOCs) or odors emitted by the food contaminants they want to detect. These could be compounds produced by bacterial pathogens like Salmonella or chemicals such as pesticides or toxins.

7.1.2. Training Phase

In the training phase, the wasps are exposed to the target odors in a controlled environment. Initially, the wasps are introduced to a clean environment with the target odour present. When they successfully detect the odour, they are rewarded with food or sugar solution. This conditions the wasps to associate the target odour with a reward.

7.1.3. Testing Phase

After the training phase, the wasps are tested to see if they can reliably detect the target odors. This usually involves presenting them with a choice between the target odour and a control odour (*e.g.*, a clean sample). If the wasps consistently choose the sample with the target odour, this demonstrates that they have successfully achieved learned to detect it.

7.1.4. Deployment

Once trained, the sniffer wasps can be deployed in various settings to detect food contaminants. This could include food processing facilities, farms, or even in the field during food production. The wasps can be placed in specially designed traps or containers that allow them to access air samples from the environment. If they encounter the target odour, they will exhibit specific behavior, such as hovering around or landing on the source of the odour.

7.1.5. Signal Interpretation

Researchers or handlers monitor the behavior of the wasps to interpret their responses. This could involve observing their movement patterns or using video surveillance to track their behavior. When the wasps recognize the presence of the target odour, their behavior serves as an indication that food contamination may be present.

7.1.6. Confirmation

While sniffer wasps can provide valuable indications of food contamination, their responses should be confirmed using traditional laboratory methods. If the wasps indicate the presence of contaminants, samples can be collected from the area where the wasps exhibited the behavior and analyzed using techniques such as microbiological testing or chemical analysis to confirm the presence of the contaminants.

7.2. Laboratories Involved in Sniffing Technology

As per current knowledge, there are four internationally recognized laboratories are actively involved in sniffing technology, especially in western honey bee *Apis mellifera viz.*, Sandia National Laboratories (SNL), The University of Montana, DARPA, Fort Leonard Wood Army base, Missouri, Air Force Research Laboratory (AFRL). Similar technology was adopted for the hymenopteran parasitic wasp *Microplitis croceipes* in two different laboratories under the University of Georgia and Los Alamos National Laboratory, New Mexico.

8. Neural Anatomy of Honeybee and Pathway of Information Transmission

Honeybees have four main sensory stations responsible for receiving and interpreting sensory stimuli: the antennae, antennal lobe, mushroom body and lateral horn. The antennae contain various types of sensilla, which are sensory hairs housing olfactory receptor neurons (ORNs). Each sensillum can host dendrites from up to four ORNs. Odorants from the air enter these sensilla through pores in the antennal cuticle, stimulating ORNs to generate specific electrical signals known as spikes in response to the odors detected. The axons of these ORNs form the antennal nerve, transmitting signals to the antennal lobe (AL), the primary olfactory processing center. The AL comprises about 165 spherical structures called glomeruli, where the ORNs make contact with approximately 4000 inhibitory local neurons (LNs) and around 800 projection neurons. These LNs perform local computations, while projection neurons relay processed information through two main tracts: the lateral antenno-cerebralis tract (l-APT) and the medial tract (m-APT). The l-APT initially projects to the lateral horn (LH) and subsequently to specific regions of the mushroom body (MB), namely the calyces (lips and basal ring). Conversely, the m-APT projects in the reverse order, from the MB calyces to the LH. Each neuron within these tracts conveys information from a single glomerulus, ensuring a precise and organized pathway for odor information processing in the honeybee brain.

The honeybee's olfactory system operates through two distinct and mostly independent subsystems, spanning from sensory inputs to higher brain centers where they project to distinct, non-overlapping regions. One subsystem involves multi-glomerular projection neurons forming the medio-lateral tract (ml-APT). These neurons transmit information directly to the medial protocerebrum and the lateral horn (LH). The mushroom bodies (MB), containing approximately 170,000 intrinsic neurons called Kenyon cells (KCs), play a central role. KC dendrites form structures known as calyces, while their axons constitute the pedunculus. The MB outputs to vertical and horizontal lobes, formed by collaterals of KC axons. Feedback neurons (FN) from the pedunculus and lobes provide inhibitory feedback to MB input regions. Extrinsic neurons (ENs) relay information from the pedunculus and lobes to various parts of the protocerebrum, notably to the LH. Descending neurons from these regions likely regulate olfactory behaviors. An octopaminergic neuron, VUM-mx1, identified in the suboesophageal ganglion (SOG), receives gustatory input from sucrose receptors and integrates with the olfactory pathway at three key areas: the antennal lobe (AL), MB calyces and LH. The mushroom bodies are crucial for olfactory memory formation, while the lateral horn serves as the terminal field for projection neuron (PN) axons involved in odour recognition.

9. Molecular Mechanism of Olfaction

On the honeybee's antenna, sensilla placodea (pore plate sensilla) are the predominant olfactory structures (Esslen and Kaissling, 1976). Each sensillum placodeum consists of an oval-shaped cuticular plate (9 µm × 6 µm) with numerous tiny pores and is innervated by five to 35 olfactory receptor neurons (ORNs) (Schneider and Steinbrecht, 1968; Esslen and Kaissling, 1976; Kelber *et al.*, 2006). Odorant molecules reach the dendrites of ORNs by diffusing through an extracellular fluid called sensillum lymph, which fills the sensillum cavity (Kaissling, 1987; Masson and Mustaparta, 1990). Odorant binding proteins (OBPs) in this fluid may assist in transporting odorants to the ORNs, although their role in bees is not well understood. Upon reaching the ORN membrane, the odorant molecules make contact with olfactory receptor proteins (ORs). Insects' ORs are a diverse family of proteins with seven transmembrane domains, distinct from vertebrate ORs (Benton, 2006; Touhara and Vosshall, 2009). The functional receptor complex includes an OR and a broadly expressed co-receptor, AmOr2 in honeybees. Within each glomerulus of the antennal lobe (AL), ORNs release acetylcholine (ACh), which is the primary excitatory neurotransmitter in the insect brain (Bicker, 1999). This activation of ORNs triggers local neurons (LNs) that connect different glomeruli, as well as projection neurons (PNs) that relay processed olfactory information from the AL to higher brain centers like the lateral horn (LH) and mushroom bodies (MBs).

The molecular mechanism of olfaction in honeybees involves a complex interplay of sensory receptors, signaling molecules and ion channels. At the forefront are odorant receptor proteins expressed on the dendritic surfaces of olfactory sensory neurons within the honeybee's antennae. These receptors are tuned to detect specific odorant molecules present in the environment. Upon binding of an odorant molecule to its corresponding receptor, a signaling cascade is initiated. This cascade involves the activation of G protein-coupled receptors (GPCRs), leading to the activation of an enzyme called adenylate cyclase. Adenylate cyclase then catalyses the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), serving as a second messenger. Elevated levels of cAMP result in the opening of cyclic nucleotide-gated ion channels (CNGCs), allowing the influx of cations such as calcium and sodium into the olfactory sensory neuron. This influx of ions depolarizes the neuron's membrane potential, generating an action potential that propagates along the neuron's axon. Ultimately, these action potentials are transmitted to the brain's olfactory centers, where they are processed and integrated to produce a perception of the sensed odour. The precise orchestration of these molecular events enables honeybees to discern and respond to a wide array of olfactory cues in their environment, crucial for tasks such as foraging, navigation and communication within the hive.

10. Sniffer Bee in Disease Detection

10.1. Lung Cancer

Sniffer bees, trained to detect the unique odour signatures linked with several diseases, including cancer, have emerged as a novel and promising tool in early diagnosis. These remarkable insects possess an exceptional olfactory sense capable of detecting volatile organic compounds (VOCs) emitted by cancer cells with remarkable accuracy. For instance, researchers at the Portuguese University of Minho have successfully trained bees to detect lung, skin and pancreatic cancers by associating the scent of cancerous cells with a sugary reward, akin to traditional Pavlovian conditioning (Rehman *et al.*, 2017). The bees' ability to discriminate between healthy and cancerous samples, even at early stages, highlights their potential as a cost-effective and non-invasive screening method. Moreover, their deployment could revolutionize healthcare in resource-constrained settings where access to advanced diagnostic technologies is limited. As published in the Journal of Chromatography B, this innovative approach underscores the intersection of biology and technology in tackling complex medical challenges. The honeybees are trained with secondary metabolites of cancer cells *viz.*, Hexanal, Heptanal, Octanal, Decanal, Dodecanal, Benzaldehyde and Phenylacetaldehyde to detect the air samples to the exhalation of patient (Parnas *et al.*, 2023).

10.1.1. Two Chambered Glass

The glass apparatus consists of two compartments: a smaller chamber designated for diagnostic testing and a larger holding chamber that temporarily houses previously trained bees. Individuals exhale into the smaller chamber and if the bees detect the specific odor they were conditioned to recognize-indicative of certain health conditions-they promptly move into the diagnostic space in response to the scent.

10.1.2. Example

UK-based product designer Susana Soares has created a simple, elegant way of harnessing bees to screen for several diseases, including cancers, like tumours of the lung and ovaries. Her glass apparatus called "Bee's," features a large chamber and a smaller connected chamber housed within it. After training the bees to associate a specific chemical odour with a food reward, such as sugar, the insects are released into the diagnostic device through an opening. Patients would simply blow into the smaller compartment and wait to see if a swarm gathers toward something alarming in the person's breath. The project, part of her master's thesis at London's Royal College of Art, began in 2007 when Soares came across research on bees and their phenomenal olfactory abilities. After talking to researchers in the field, she learned that certain diseases, such as lung cancer, noticeably alter the composition of bodily fluids, producing odorous compounds that show up in urine and sometimes blood.

10.2. Diabetes

Acetone is one of the key volatile organic compounds (VOCs) found in the breath of diabetes patients, especially those experiencing uncontrolled or poorly managed diabetes. The presence of acetone in the breath is primarily attributed to the metabolic changes that occur in individuals with diabetes, particularly in cases of diabetic ketoacidosis (DKA), a serious complication of diabetes characterized by increased levels of ketone in the blood. In diabetes, when there is insufficient insulin to facilitate glucose uptake by cells is impaired, the body compensates by metabolizing fats to meet its energy needs. This process leads to the production of ketones, including acetone, as byproducts of fat metabolism. Acetone is produced when acetoacetate, one type of ketone bodies, is further metabolized. These results in elevated levels of acetone in the breath can serve as a marker for DKA and can indicate inadequate insulin control or other disruptions in metabolism related to diabetes. Breath acetone measurements can therefore be used clinically as a non-invasive method for monitoring diabetes management and assessing the risk of DKA. The mechanism of acetone production in diabetes breath air underscores the metabolic alterations that occur in the absence of adequate insulin action and highlights the potential utility of breath analysis as a diagnostic and monitoring tool in diabetes care.

Sniffer bees could potentially be trained to detect diabetes in individuals by recognizing the unique odour signatures linked to secondary metabolites, including acetone in the breath of diabetes patients (Ahmed and Anwar, 2014). Acetone is a volatile organic compound (VOC) that is elevated in the breath of individuals with uncontrolled or poorly managed diabetes, particularly in cases of diabetic ketoacidosis (DKA). The training process for sniffer bees involves conditioning them to link the odor of scent of specific VOCs, like acetone, with a reward. In this case, bees can be trained to recognize the distinctive scent profile of acetone using a similar Pavlovian conditioning technique as utilized in cancer detection studies. Bees would be exposed to breath samples from diabetes patients containing acetone and upon correctly identifying these samples; they would receive a reward, such as sugar water. Through repeated exposure and reinforcement, sniffer bees could learn to distinguish between breath samples from diabetes patients with elevated acetone levels and those from healthy individuals. The bees' remarkable olfactory abilities make them well-suited for detecting subtle differences in odour profiles, potentially enabling them to serve as a non-invasive and cost-effective screening tool for diabetes. However, it's important to acknowledge that, despite the potential of this concept, additional research is necessary to validate the efficacy and reliability of using sniffer bees for diabetes detection based on acetone and other VOCs in the breath. Additionally, practical considerations such as standardization of training protocols and deployment methods must be thoroughly evaluated prior to clinical application of this approach.

10.3. Sniffer Bee as SARS-CoV-2 Detector

Training bees to identify SARS-CoV-2 infected samples in minks could be approached similarly to training them for other scent-based tasks, with some adaptations to accommodate the specific challenges and requirements of working with mink samples (Kontos *et al.*, 2022).

10.3.1. Sample Collection and Preparation

Samples from SARS-CoV-2 infected minks would be collected; ensuring appropriate safety protocols are strictly adhered to handle potentially infectious materials. These samples might include swabs from nasal or oral cavities, faecal samples, or other bodily fluids known to contain viral particles or related volatile organic compounds (VOCs).

10.3.2. Training Setup

Bees would be exposed to the scent of SARS-CoV-2 infected mink samples in a controlled environment. This exposure would be paired with a reward, such as sugar water, to reinforce the association between the scent and the reward. The training protocol must be tailored to the unique properties of mink samples while prioritizing the safety of both the bees and their handlers.

10.3.3. Training Protocol

The training protocol would involve repeated exposure sessions, gradually increasing in complexity as the bees develop greater expertise in recognizing the target scent. Training sessions may also include exposure to non-infected mink samples or samples from other species to ensure specificity.

10.3.4. Testing Phase

Once trained, the bees would undergo testing to evaluate their ability to discriminate between SARS-CoV-2 infected mink samples and non-infected samples. Testing would involve presenting the bees with a variety of samples in a randomized manner and recording their responses.

10.3.5. Validation and Refinement

The performance of the trained bees would be validated through rigorous testing and statistical analysis. Researchers would refine the training protocol as needed to improve accuracy and reliability.

10.3.6. Deployment

If successful, trained bees could be deployed in mink farms or other settings where SARS-CoV-2 surveillance in minks is necessary. They could serve as a rapid and non-invasive screening tool to identify infected individuals and mitigate the transmission of the virus within mink populations.

11. Comparison of Sniffer Bee and Sniffer Dog

Demining dogs are essential assets in landmine clearance due to their ability to detect explosive scents like TNT, often outperforming mechanical sensors in sensitivity. Working closely with handlers, they scan minefields and indicate a potential detection by sitting. Handlers then verify the alert using manual tools. Despite their usefulness, several challenges limit their deployment. Variability in training and handler skill can affect detection accuracy. Environmental conditions such as temperature, humidity and terrain further complicate operations. Additionally, the risk of mine activation due to a dog's weight, the need for one-at-a-time deployment and rapid fatigue make large-scale or prolonged use difficult (Table 1).

Table 1: Difference between sniffer dogs and sniffer bee

Comparison	Sniffer Dogs	Sniffer Bees
Training time	6 months	6 seconds
Operational time	8 hours shift per dog	24/7: continuous supplies of freshly trained bees.
Scalable	1 dog one handler	1 automatic machine = 2000 bees per day

Comparison	Sniffer Dogs	Sniffer Bees
Scientific & repeatable	Quality dependable on the human trainer	Automated, precise and repeatable process. No human errors. Quality control is part of the training process.
Cost	Dog-handler team $ 100,000, 57% specialist handler, 27% maintenance, 16% training	Very cost-effective, no specialist handler required, low maintenance, mass production of trained bees.
Triggering of landmine	The weight of a dog can trigger a landmine	The weight of the bee is not sufficient to trigger a landmine.

12. Future Prospects

12.1. Enhanced Agricultural Practices

• Early detection of pests and diseases in crops.

• Reduction in pesticide usage through targeted interventions.

• Increased crop yields and sustainability in agriculture.

12.2. Environmental Monitoring and Protection

• Real-time detection of pollutants and toxins in the environment.

• Monitoring of air and water quality for conservation efforts.

• Rapid response to environmental threats for ecosystem preservation.

12.3. Search and Rescue Operations

• Assistance in locating missing persons in challenging terrain.

• Efficient search capabilities in disaster zones or wilderness areas.

• Improved search accuracy and speed compared to traditional methods.

12.4. Security and Law Enforcement

• Detection of explosives, narcotics and contraband materials.

• Strengthening security measures at airports, border crossings and public events.

• Combating illegal activities through enhanced detection capabilities.

12.5. Medical Diagnostics and Healthcare

• Potential for early detection of diseases and infections based on scent markers.

• Non-invasive diagnostic tool for medical screening and monitoring.

• Advancements in personalized medicine and treatment strategies.

12.6. Urban Planning and Pest Management

• Identification of pest infestations and environmental hazards in urban areas.

• Support for urban planning initiatives and sustainable city development.

• Integrated pest control techniques for maintaining public health and safety.

12.7. Conservation and Biodiversity Conservation

• Monitoring of endangered species and their habitats through scent detection.

• Support for conservation efforts and wildlife management.

• Preservation of biodiversity and ecosystems for future generations.

13. Conclusion

The application of sniffer bees in military and defense sectors offers a paradigm shift in detection capabilities. Their innate ability to detect explosives, narcotics and other contraband materials presents a non-invasive and an efficient technique for enhancing security measures at airports, border crossings and military installations. Furthermore, in disease detection, sniffer bees showcase immense potential for early diagnosis of various medical conditions through scent markers, revolutionizing healthcare practices with their rapid and accurate detection capabilities. Additionally, in the realm of food safety, sniffer bees offer a novel approach to identifying contaminants and pathogens in food products, thereby safeguarding public health and minimizing the risk of food-borne illnesses. By harnessing the remarkable olfactory prowess of these tiny yet powerful creatures, industries and institutions can benefit from cost-effective, environmentally friendly and reliable solutions for a wide range of detection and surveillance challenges. As research and technological advancements continue to expand our understanding of sniffer bee capabilities, their integration into military, defense, disease detection and food safety protocols have offers substantial promise for addressing pressing societal needs with efficiency and precision.

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