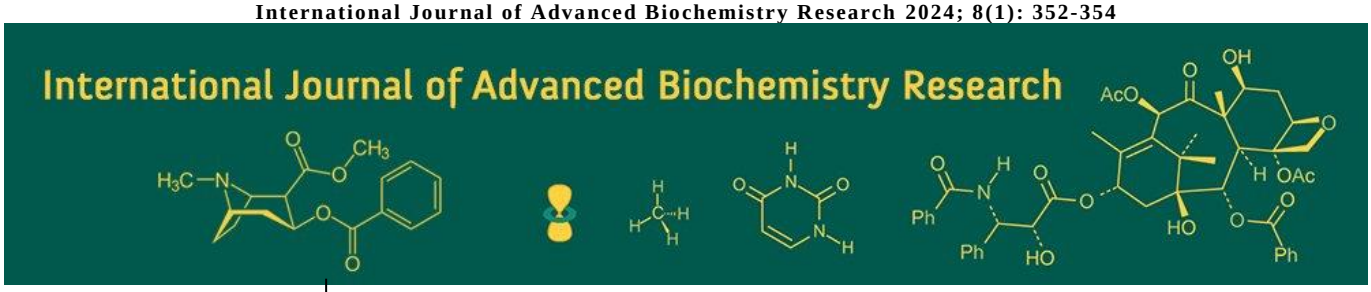

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Antifungal activity of raw honey collected from *Apis dorsata* Fabricius Honeybee

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Abstract
The ingredients of honey have been reported to exert antioxidant, antimicrobial, anti-inflammatory, anti-proliferative, anticancer, and anti-metastatic effects. The present investigation aimed to study the antifungal activity of raw honey of three honeybee species collected from the Kumaon region of Uttarakhand. Honey samples were collected and assayed against *Fusarium oxysporum* fungal species by Kirby–Bauer disk diffusion technique. Among all the honey samples. *Apis dorsata* honey was effective against the *Fusarium oxysporum.* The inhibition zone clearly showed that honey obtained from raw *Apis dorsata* had the highest antifungal activity, while honey samples from other species showed no antifungal activity.

Keywords: Honey, *Apis dorsata*, inhibition zones, antifungal activity

Introduction
The definition of honey is "a sweet, viscous natural fluid substance made by honeybees from blossom nectar, which the bees gather, process, and store in structures resembling hexagons of wax called honeycombs (Codex Alimentarius 2001) [1]. According to scientific data, honey has significant biochemical therapeutic potential and can treat a variety of illnesses. Molan, (1996) [2]. Numerous research demonstrated honey's greater activity compared to several well-known antibiotics (Al-Waili and Saloam 1999) [3]. The antibacterial properties of honey were found to be potent against both pathogenic and non-pathogenic microorganisms. Molan, (1996) [2]. According to his past studies, honey possesses antibacterial qualities and can expedite the healing process of human ailments. Therefore, honey can eradicate all surgical site germs and manage wound infections following surgery that are brought on by different types of bacteria. Strong antibacterial qualities found in honey can be used safely and affordably (Fessenden R.E, 2008) [4]. Various studies have reported the antimicrobial activity of honey (Agbaje *et al*., 2006) [5]. Aflatoxin B1 and B2 levels are lowered and *Aspergillus flavus* growth is inhibited by honey. It has been observed that the inherent qualities of honey exert bacteriostatic effects on the growth and survival of bacteria (Iurlina, MO & Fritz R, 2005; Mekky, 2007) [6, 7]. Its antifungal action has been observed against the yeast *Candida albicans* and most species of *Aspergillus baumannii* as well as *Penicillium chrysogenum* and all the common dermatophytes (Willix *et al*., 1992; Brady *et al*., 1997) [8, 9]. In addition, the antifungal effect of honey from different regions has been shown in different studies, on different strains such as *Aspergillus spp., Alterneria spp., Fusarium spp., Microsporum spp., Penicillium spp., Rhizopus spp. or Candida spp*. (Ceyhan, 2001& Ahmad, 2017) [10, 11].

In recent years, there has been an increasing search for new antifungal compounds due to the lack of efficacy, side effects and or resistance associated with some of the existing drugs. Therefore, honey could be one of the best alternative organic antimicrobial agents to treat pathogens (Barker and Rogers 2006; Ali *et al*., 2010) [18, 19].

Materials and Methods
To evaluate the antifungal potential of honey, samples were taken from three different kinds of honeybees: *Apis mellifera* (raw), *Apis cerana* (raw), and *Apis dorsata* (raw) and each sample were replicated three time, The Kirby-Bauer diffusion technique was used to test the

antagonistic potential against phytopathogenic fungi *viz*, *Fusarium oxysporum for which* PDA medium plates were prepared. The actively grown fungal disc (5 mm diameter) was placed centrally in a PDA medium plate then raw honey samples were inoculated well towards the periphery of the Petri plate and incubated for 5–6 days at 25 ± 2 °C. The control plates were maintained by adding distilled water instead of honey inoculation. Appearance of zone of inhibition in fungal growth can be seen in Figure-1.

Results and Discussion

The pathogenic *Fusarium oxysporum* fungi, which are known to cause head blight (FHB) in several crops, including canary seed, wheat, barley, oats, rye, corn, triticale, and some forage grasses, were tested for their antifungal properties against all three honey samples (Alisaac and Mahlein, 2023) [12]. The only honey found to be able to stop *Fusarium oxysporum*'s mycelia from growing was *Apis dorsata* honey. The antifungal property of the honey from *Apis mellifera* and *Apis cerana* was not reported since it allowed *Fusarium oxysporum* to grow mycelia past their inhibitory effects. Numerous studies have found that honey has a range of bioactive ingredients that prevent phytopathogens from growing in crops when they are in the mycelial stage. These include phenolic substances like flavonoids and phenolic acids, as well as other polyphenols, methylglyoxal (MGO), and the inherent acidity of honey. Hydrogen peroxide is created by the enzyme glucose oxidase. Furthermore, honey's defenses against microbes are strengthened by bee-derived peptides and proteins, such as bee defensin-1. The combined activity of these several chemicals highlights the efficacy of honey as a natural antifungal agent (Molan, 1992). The current investigation supports the results of Moussa *et al*., (2011) [13] who looked at the antifungal qualities of four Algerian honeys made from different botanical sources against *Rhodotorula sp*. and *Candida albicans*. The findings of their experiment confirmed the antifungal activity of all honey samples against *C. albicans* and *Rhodotorula sp*. by showing that all honey samples suppressed the mycelial growth in both fungi. Cavanagh *et al*., (1970) [14] found that a 100% (v/v) concentration of honey had a complete fungicidal effect on *C. albicans*, while a 50% concentration was necessary to bring about the same action in the species of *C. stellatoidea, C. reukaufii, and C. tropicalis.* A similar kind of activity was shown by the *Apis dorsata* honey. Our findings were also in agreement with Molan's review of the antimicrobial activity of honey. Anand *et al*., (2019) [15] also studied the antifungal activity of mono-foral *Agastache* honey and commercially available honeys were tested against dermatophytes (*T. mentagrophytes and T. rubrum*) and *C. albicans* (ATCC 10231 and a clinical isolate), *Agastache* honey was more effective as compared to the other commercial at 40% concentration against dermatophytes similarly raw *Apis dorsata* honey was more effective as antifungal agent. Groot *et al.,* (2021) [16] also revealed that medical-grade honey effective against the fungus *Candida auris.* However, different results were obtained by Bhalchandra *et al.,* (2022) [17] where zone of inhibition clearly showed that honey obtained from *Apis florea* and *Apis cerana indica* had highest antifungal activity, while a honey sample of *Apis dorsata* showed minimum antifungal activity.

Fig 1: Antifungal activity of *Apis dorsata* honey against *Fusarium oxysporum*

Conclusion

Overall, this study highlights the significance and potential uses of raw *Apis dorsata* honey in alternative medicine and healthcare by confirming its antifungal potential. Hence this research gives evidence that raw honey of *Apis dorsata* could play a potential therapeutic role. It could be one of the best organic alternatives to traditional drugs and fungicides in the treatment of disease caused by inflammatory and antimicrobial agents.

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