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Antifungal Activity of Raw Honey of Three Honeybee Species Harvested from Kannad Taluka of Aurangabad District (M. S.), India

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Abstract: ***The aim of present investigation was to study the antifungal activity of raw honey of three honeybee species collected from Kannad taluka of Aurangabad district. Honey samples were collected and assayed against three fungal species by using Agar well diffusion method. All honey samples showed sensitivity against all the fungal species. The zone of inhibition clearly showed that honey obtained from Apis florea and Apis cerana indica having highest antifungal activity, while honey sample of Apis dorsata showed minimum antifungal activity. The most sensitive fungi were Aspergillus and Fusarium, while Alternaria spp. was less sensitive to the inhibitory action of honey.***
Keywords: Honey, Apis dorsata, Apis florea, Apis cerana indica, zone of inhibition, antifungal activity, sensitivity, etc.

I. INTRODUCTION

Honey is defined as a sweet viscous natural fluid substance produced by honeybees from the nectar of blossoms, which the bees collect, transform and store in hexagonal wax like structure known as honey combs [1].

Scientific reports showed that honey exhibits important biochemical therapeutic activity as it cures various ailments [2]. Several studies showed the higher activity of honey over many known antibiotics [3]. Honey showed powerful antibacterial effect against pathogenic and non-pathogenic microorganism. [2] Molan (1996) in his earlier research reported that honey can accelerate man diseases healing activity and also had bactericidal properties. Honey is thus able to destroy all of the bacteria that cause surgical infections and also able to control post-operative wound infections caused by various bacterial species.

[4] Bashir (2009) reported, honey inhibits the growth of a wide range of microbes. Honey has several important properties and the sugar solution of honey has high osmolarity, which inhibits microfloral growth. Thus, the natural acidity of honey inhibits many pathogenic organisms.

The past two decades have brought a resurgence of interest in learning more about antimicrobial activity. Mould, yeasts and spore forming bacteria are primarily the microbes of great concern in honey. Microbiological characteristics of honey are inherent to quality and safety [5]. Certain fungi that can grow on food such as dried fruits, nuts, cereals, legumes and spices produces naturally-occurring toxins called mycotoxins. The most commonly observed mycotoxins are found aflatoxin (B1, B2, G1 and G2) and ochratoxin-A [6].

The use of honey as a traditional medicine for microbial infections dates back to ancient times. Its capability to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature [7]. Honey has wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location and flower from which the final product is obtained [8].

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is decreased. This type of bacterial resistance to the antimicrobial agents constitutes a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide [9, 10].

Honey possesses powerful antimicrobial properties that can be utilized at low cost and at no risk [11]. Various studies have reported the antimicrobial activity of honey [12]. Honey inhibits the growth of *Aspergillus flavus* and reduces aflatoxin B1 and B2 levels. The intrinsic properties of honey have been reported to affect the growth and survival of microorganisms by bacteriostatic actions [13, 14]. Its antifungal action has been observed against the yeast *Candida albicans* and most species of *Aspergillus baumannii* as well as *Penicillium chrysogenum* [15] and all the common dermatophytes also [16].

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Recently, honey has attracted attention within scientific community due to its potent antifungal activity [17, 18, 19]. Several researches on antifungal activity of honey had been reported against yeast *Candida albicans, Candida krusei, Cryptococcus neoformans, Aspergillus baumannii* and *Penicillium chrysogenum* as well as other common dermatophytes [20, 21].

Both the in vitro and in vivo studies have demonstrated that honey is an effective, broad spectrum and active antimicrobial agent against a wide variety of bacteria and fungi [22]. Several studies have investigated the antimicrobial properties of honey against bacteria; few have focused on its antifungal properties [23].

In recent years, there has been an escalating trend of fungal resistance to the current antifungal drugs accompanied with lack of efficacy and side effects. Thus, this fact has driven the research towards the study of antifungal agents from natural resources including honey [24].

Therefore the present study deals with the study of antifungal activity of raw honey collected from three different bee species of Kannad taluka of Aurangabad district (M. S.) India.

II. MATERIALS AND METHODS

1) *Sample Collection:* Honey samples were collected from three different honeybee species namely *Apis dorsata, Apis cerana indica* and *Apis florea* from Kannad region of Aurangabad district. These samples were assayed against three different fungal species viz. *Aspergillus, Fusarium* and *Alternaria spp.*
2) *Antifungal Activities:* The screening of antimicrobial activity of each honey sample (1:1) on the tested pathogens used in this investigation was determined on Potato Dextrose agar media (all tested organism grow on Potato Dextrose agar media), by the using agar well diffusion method [25, 26]**.** Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. Approximately 20µl of each extract was inoculated onto wells were made in the spread plate culture of each microbial isolates. The plates were performed in triplicates. All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 hours of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm) and represented as, +++ = Maximum antifungal activity, ++ = Average antifungal activity and + = Minimum antifungal activity. In this study Fluconazole was used as a standard antibiotic.

III. RESULTS AND DISCUSSION

Several *in vitro* studies have demonstrated the antibacterial properties of honey [27, 28, 29] few have examined the action against fungi. The incidence of fungal infections is increasing in both the community and hospital environments with several causative agents including yeasts with *Candida* spp., among the leading organisms and filamentous fungi [30]. In the present study the antifungal activity of honey of three bee species collected from Kannad taluka of Aurangabad district were determined and obtained results are presented in Table No.1 and Plate-II.

Results indicated that honey samples of three bee species showing the antifungal activity against three fungal strains (*Aspergillus, Alternaria* and *Fusarium spp.*). It was observed that *Apis cerana indica* and *Apis florea* honey shows the highest antifungal activity on three studied fungal strains than *Apis dorsata* honey.

The honey harvested from *Apis florea* showing the average antifungal activity (++) against the fungal strain like, *Aspergillus* and *Fusarium* species and the minimum antifungal activity (+) against *Alternaria spp.*

The honey harvested from *Apis dorsata* showing the average antifungal activity (++) against the fungal strain like, *Aspergillus spp.* and the minimum antifungal activity (+) against *Alternaria* and *Fusarium spp.*

The honey harvested from *Apis cerana indica* showing the average antifungal activity (++) against all the three fungal strain like, *Aspergillus, Alternaria* and *Fusarium spp.* Results also demonstrate that *Aspergillus* and *Fusarium spp.* were the most sensitive spp. while *Alternaria spp.* was less sensitive to the inhibitory action of honey.

[20] Anyanwu (2012) studied the *in vitro* antifungal activity of honey obtained from different geographical locations in Nigeria against some fungal isolates (*Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Microsporum gypseum, Candida albicans* and *Saccharomyces* species) and reported that the honey samples show varying level of inhibitory activity at various concentrations against the tested fungi with zone of inhibition increasing with increasing the honey concentration (w/v). [31] Nik et al., (2016) determined the antifungal activity of Malaysian honey and propolis extracts against pathogens implicated (*Candida spp.*) in denture stomatitis and reported that honey produced from *Trigona spp.* had strong antifungal activity as compare to *Apis dorsata* and *Apis mellifera* honey against oral fungi implicated in denture stomatitis as well as reported the zone of inhibition ranges between 8.69 to 12.29 mm.

Several factors may influence the antifungal activity of honey. These factors include its physico-chemical properties, botanical origin and entomological origin. The ability of the honey samples to inhibit the growth of several fungal species is an indication of the broad-spectrum antifungal potential of the honey which makes it a candidate for application as an antifungal agent [20].

[32] Sayadi et al., (2015) studied comparative antifungal activity of selected Malaysian honeys and Manuka honey and reported that all tested honeys (except Gelam) demonstrated inhibitory effects against the tested species.

[33] Fernandes et al., (2021) studied Portuguese honeys as antimicrobial agents against *Candida spp.* and found that all honeys at 50% (w/v) induced inhibition of a range of pathogenic *Candida spp.* in planktonic state as well as significant difference was observed among the candidacidal activities of all honeys tested.

IV. CONCLUSION

The *Aspergillus* and *Fusarium* were the most sensitive fungi *spp*. while *Alternaria spp*. was less sensitive to the inhibitory action of honey. It was also clear that, *Apis florea* and *Apis cerana indica* honey showing the maximum antifungal activity on three studied fungal strains in comparison with the honey sample obtained from *Apis dorsata.* It was concluded that the honey obtained from three bee species from Kannad taluka are good in antifungal activity.

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Table No. 1: Antifungal activities of honey samples of three honey bee species.

Sr. No.	Fungal strains	*Apis florea* honey	*Apis dorsata* honey	*Apis cerana indica* honey
1	*Aspergillus spp.*	++	++	++
2	*Alternaria spp.*	+	+	++
3	*Fusarium spp.*	++	+	++

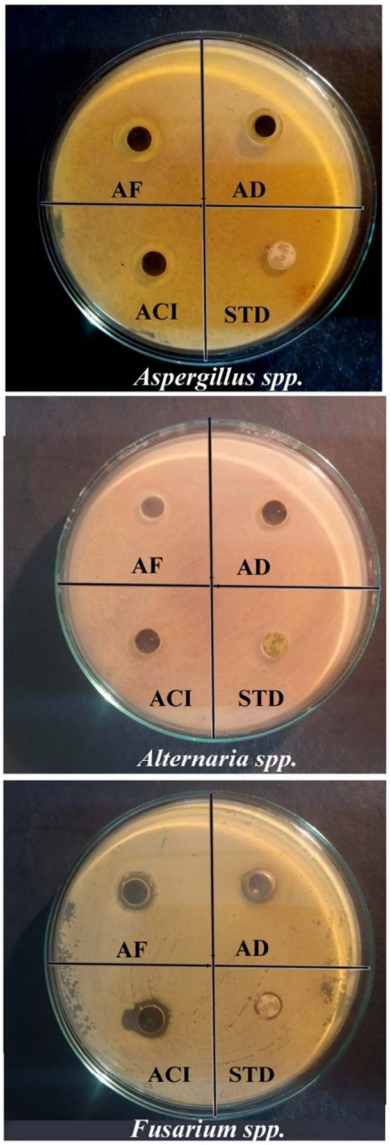
+++ = Maximum antifungal activity, ++ =Average antifungal activity and + = Minimum antifungal activity.

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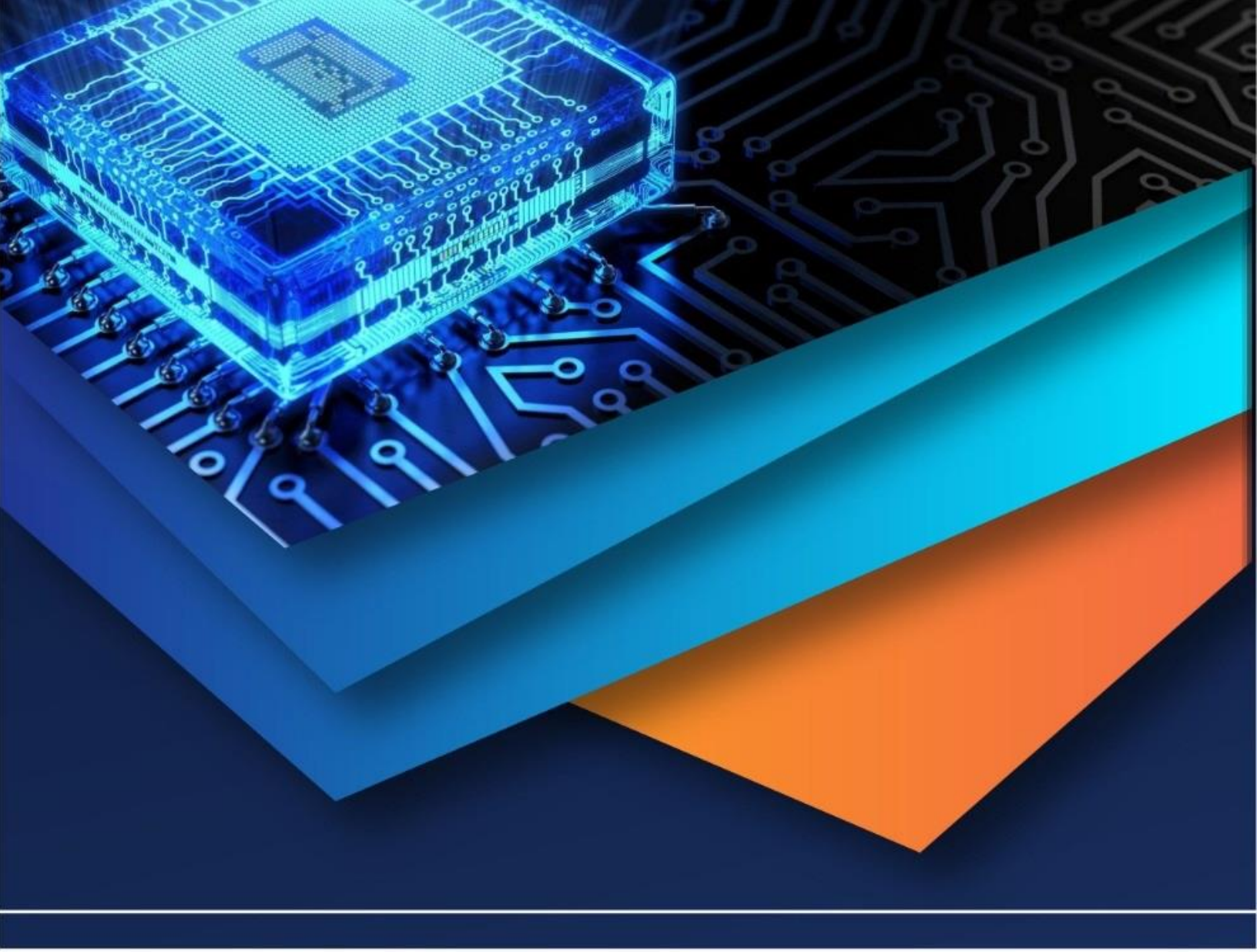
PLATE - II

Antifungal activity of honey of three honeybee species from Kannad taluka



Abbrevations : AF - *Apis florea* , AD - *Apis dorsata*, ACI - *Apis cerana indica* and STD - Standard

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