STUDY ON THE EFFECT OF PROPOLIS ON THE BEE HEALTH

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July, 2016

Dedicated to
My Beloved Parents

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CERTIFICATE

This is to certify that the thesis entitled, **"STUDY ON THE EFFECT OF PROPOLIS ON THE BEE HEALTH.''** submitted to the Department of Entomology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) IN ENTOMOLOGY,** embodies the result of a piece of bonafide research work carried out by NAME**MD. SAMSUL ALAM** bearing **Registration No. 10-04162**under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institution elsewhere.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 18.05.2016
Place: Dhaka, Bangladesh

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ACKNOWLEDGEMENTS

The author wishes to acknowledge the immeasurable grace and profound kindness of the "Almighty Allah" the Most Gracious and The Supreme Rule of the universe for giving mental peace, health and strength to submit the thesis for the degree of Master of Science (MS) in Entomology.

*The author would like to extend his heart-squeezed gratitude, deepest appreciation, best regards and indebtedness to his honorable teacher and research Supervisor **Prof. Dr. Mohammed Sakhawat Hossain,** Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar Dhaka, for his untiring guidance, scholastic supervision, valuable advice, innovative suggestions, constant encouragement, helpful comment, affectionate feeling and inspiration in all phases of conducting the research work and preparation of the thesis.*

*The author would like to express his sincere appreciation, heartfelt gratitude and immense indebtedness to his research Co-supervisor **Prof. Dr. Mohammed Ali**, Department of Agronomy, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar Dhaka, for his kind cooperation, encouragement, affectionate feelings, technical help, valuable advice and helpful discussion throughout the entire period of research work and preparation of the thesis.*

*The author would like to express gratitude to, Professor, **Prof.Dr. Md. Mizanur Rahaman**, Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar Dhaka. The author feels proud to express and boundless indebtedness to all the honorable course teachers.*

Cheerful acknowledgements are expressed to his younger brother Md. Shafiqul Alam who helped him with financial support to prepare this thesis paper. The author would like to thank his friend Md. Arifur Rahman, Md. Yeasinul Hoq, Md. Tazul Islam polas, Md.Mohiuddin Fozlullah . Besides special thanks to lab assistant Md. Mahbubur Rahman And Nazmul Haq who helped him with technical support to prepare this thesis paper. The author wishes to extend his special thanks to his supervisor Pro. Dr. Mohammed Sakhawat Hossaine who Helped him by giving opportunity to work his project to run smoothly the research activities.

Finally the author is ever grateful to his respective parents Md. Hebzul Bari And Late Minara Begum for their everlasting love, patience, moral and constant blessings.

The Experiment was carried out under a research project funded by Krishi Gobeshona Foundation (KGF). We acknowledged their funding to complete thesis paper.

July, 2016 ***The Author***

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ABSTRACT

An experiment was conducted in different beekeepers Apiary of Gazipur, Satkhira and Sirajgonj districts of Bangladesh during the period from November 2015 to May 2016 to determine the efficiency of propolis yield and the effect of propolis on the bee health. Paired plot techniques was used to reveal the traditional versus polyhive type pollen yield in different sites. It was observed that the average propolis yield using Traditional hive were nil whereas, using Super hives gives varying yields i.e. Sirajganj district 17.22g propolis, Gazipur 25.31g and Shatkhira, 26.03g of propolis. Bee health was also observed in the propolis yielded hive versus traditional hive. In each case have poly hive pests and diseases attack were low in comparison to traditional ones. However, there was no American foul brood, Sac brood, Chalk brood and Deformed winged virus diseases during the experimental period in both the treatments.

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SOME COMMONLY USED ABBREVIATION

FULL WORD	ABBREVIATION
And others	*et al.*
Bangladesh Bureau of Statistics	BBS
Bangladesh Agriculture and Research Institution	BARI
Cultivar	cv.
Degree Celcius	^{o}C
edest (means That is)	i.e.
Figure	Fig.
Gram	g
Micro gram	µg
Micro mol	µM
Milligram/litre	mgL^{-1}
Namely	Viz.
Parts/million	ppm
Percentage	%
Species (plural number)	spp.
Variety	var.

CHAPTER I

INTRODUCTION

Propolis or bee glue is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. The collection begins when an expert propolis-making bee gathers resin from cone-producing evergreen trees or from the buds of trees. The bee will gather this sticky sap when the proper weather makes it pliable and soft. After the bee gathers enough, they blend resin with wax flakes that he store in the gland of his abdomen. After shaping it into a ball, they tuck it into the pollen basket that is attached to thier leg. Bees continue the procedure until the basket is full, then take it back to their hive. In the hive they unload propolis inthe hive and patch up holes of the hive. propolis is used to seal the cracks, to make smooth walls, and to keep moisture and temperature stable in the hive all the year around.As a result the hive remains safe and sound. Consequently production of bee products like honey , wax, royal jellyetc increases. Honey bee resin foragers follow a fairly strict diurnal pattern in foraging and also cementing as well as behaviors. Foraging for resins is typically observed between 10 am to 3:30pm in sunny days, increased pliability of resins at higher temperatures enhances foraging for rasins(Alfonsus, 1933; Meyer, 1956; Hoyt, 1965; Nyeko*et al.*, 2002). Cementing behavior occurs most often in late afternoon by the foragers. Oncetheir loads have been removed (Meyer, 1956; Ratnieks and Anderson, 1999).Additionally resin foragers almost always be found from May through November in temperate regions (Crane, 1990), there appears to be some seasonality in resin collection and propolis use. Resin is said to be collected most frequently in late summer (end of June) through autumn when the honey flow is greatly reduced (Alfonsus,1933; Meyer, 1956; Crane, 1990). The composition of propolis varies from hive to hive, from district to district, and from season to season. "Typical" northern temperate propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%),waxes (30%), essential oils (10%), and pollen (5%). Propolis also contains persistent lipophilic acaricides, a natural pesticide that deters mite infestations. From a systematic database research, 241compounds were identified in propolis for the first time between 2000 and 2012; and they belong to such diverse chemical including flavonoids, terpenes, phenolics and their esters, sugar, hydrocarbon and mineral element.

Propolis has a long history of medicinal use, dating back to 350 B.C., the time of Aristotle. Greeks have used propolis for abscesses; Assyrians have used it for healing wounds and tumors; and Egyptians have used it for mummification. It still has many medicinal uses today. Propolis is used for canker sores and infections caused by bacteria (including tuberculosis), by viruses (including flu, H1N1 "swine" flu), and the common cold, by fungus, and by single-celled organisms called protozoans. Propolis is also used for cancer of the nose and throat; for boosting the immune system; and for treating gastrointestinal (GI) problems including Helicobacter pylori infection in peptic ulcerdisease. Propolis is also used as an antioxidant and anti-inflammatory agent. People sometimes apply propolis directly to the skin for wound cleansing, genital herpes and cold sores; as a mouth rinse for speeding healing following oral surgery; and for the treatment of minor burns. In manufacturing, propolis is used as an ingredient in cosmetics. The vast majority of propolis collecting devices in the world is based on bee's instinct to fill gaps and openings in the hive smaller than 4mm. The amount of this product in the hive is variable and depends on many factors: bee breed, geographic and climate conditions, hive type, presence of propolis source in nature, strength of a bee colony. The amount of propolis collected from one hive also varies. Some sources from literature state that one can collect 50 – 100g, while others mention 150 – 200g. Some beekeepers think that one colony may produce 400g of propolis and that this amount can be increased to 2kg and more using special procedures. Taking into account the causes which incite bees to collect propolis, it is possible to increase the amount of this with success. It is favourable to have increased ventilation in the hive, uneven surfaces of cover boards and hive walls, special types of hive entrance bars, and some sort of teasers for bees. Propolis collecting frames with wooden or plastic bars is often used in practice (Bankova&Marcucci, 2000). These bars make temporary gaps: 3 – 4 mm wide, which make it possible to get 250 – 400g of pure propolis in one season. The bars are put over hive frames while cover boards and all isolating material are removed. In 6 – 7 days bees fill the gaps between bars with propolis. Bars are replaced with new ones when filled with propolis. Propolis is obtained from the bars by following some steps .Bars are put in a rough cloth, rolls in so that the bars are inside, and put in a deep freezer for several hours. After that, they are taken out and unrolled with bars down, so that propolis falls down on the table. P. P. Lakers recommended a mesh with bars on different heights which are linked with each other, which makes it possible to get 1kg of propolis. The mesh is also put over hive frames instead of cover board and isolating material. Occasionally, worker bees gather various compounds of human manufacture, when the usual sources are more difficult to obtain. The properties of the propolis depend on the exact sources used by each individual hive; therefore any potential medicinal properties that may be present in one hive's propolis may be absent from another's, or from another sample in the same hive. Propolis should not be warmed, washed or melted since it loses a part of its characteristics. It should be kept in a dark place in polyethylene bags. Propolis is very stable matter, so it can safely be used up to 10 years from the day of obtaining. Foragers working outside the beehive are usually specialized to harvest particular material to increase their work efficiency. It is demonstrated that 60% of them are necter foragers, 25% are busy with pollen collecting and 15% are involved in propolis collecting (Burdock, 1998).

The objectives of the proposed study is-

- to collect bee propolis from three different districts by using traditional and polyhive type beehives
- to find out the effect of harvesting propolis on the health of bee colony

CHAPTER II

REVIEW OF LITERATURE

Honey bees (Apismellifera L.), like many other social insects, have collective behavioral defenses called "social immunity" to help defend and protect the colony against pathogens and parasites. One of the example is social immunity: the collection of plant resins and the placement of the resins on the interior walls of their nest cavity, where it is called a propolis envelope. Propolis is known to have many antimicrobial proprieties against bacteria, fungi, and viruses and has been harvesting from bee hives for use in human medicine since antiquity. However the benefit of propolis to honey bees has been studied a little.It is common knowledge that honey bees forage for pollen, nectar and water. What is not well appreciated is that honey bees also forage for plant resins, but not for nutritional reasons. Resin is a sticky exudate secreted prophylactically by plants to protect young leaf buds or the entire plant from disease, UV light, and herbivore attack (Langenheim, 2003). Resins are composed primarily of antimicrobial compounds (e.g. monoterpenes and flavonoids) that play a major role in defense and survival of the plant (Langenheim, 2003).Honey bees harvest resins from various plant species and bring them tothier the colony and use as propolis (propolis is an apicultural term for the resinswhen used by bees within a hive). The harvesting of antimicrobial compounds (resins)from the environment and their incorporation into the social nest architecture as propolisis an exciting nature procedure its use for but colony-level defense against pathogens are relatively unexplored. Much ofthe current literature concerning propolis has focused on the chemical constituents andbiological activity of propolis and the botanical origins of the resins from which thepropolis mixtures are derived (see Banskota*et al.*, 2001; Bankova*et al.*, 2008). Althoughthis work is certainly interesting due to the pharmacological benefits to human , we still remain largely unaware ofthe benefits of resin collection to honey bees and the basic mechanisms that drive resinforaging at both the individual and colony levels. This review provides a compilation ofrecent research concerning the behavior of bees in relation to resins and propolis,focusing more on bees themselves and potential evolutionary benefits of resin collectionand not on chemical analyses of propolis and resins or implications for human health.

Benefits of propolis to colony health: It is common knowledge that honey bees forage for pollen, nectar and water. What is not well appreciated is that honey bees also forage for plant resins, but not for nutritional reasons. Resin is a sticky exudate secreted prophylactically by plants to protect young leaf buds or the entire plant from disease, UV light, and herbivore attack (Langenheim, 2003). Resins are composed primarily of antimicrobial compounds (e.g. monoterpenes and flavonoids) that play a major role in the defense and survival of the plant (Langenheim, 2003). Our research has found that these antimicrobial resins also play a major role in the immune defense and health of honey bee colonies.Honey bees collect resin mainly from buds and leaves of various tree species, but they also collect resins from droplets appearing on the trunks or limbs of trees (Alfonsus, 1933), from the surfaces of some fruits (e.g., Macarangatanarius; Kumazawa *et al.*, 2003), or as a reward for pollination of some flowers [e.g., Clusia (Clusiaceae) andDalechampia (Euphorbiaceae)] (Armbruster, 1984). Bees can extract resin by fragmenting leaves with their mandibles (mouthparts) or collect it directly from the plant surface (Meyer, 1956; Teixeira *et al*., 2005). Bees collect resins to varying degrees; some honey bee species and races use resins extensively; for example African-derived subspecies Apismelliferascutellata, and European-derived subspecies *A. melliferacaucasica*. At least one species of honey bees, *Apiscerana*, is reported to collect no resin (Butler, 1949; Page and Fondrk, 1995). In colonies that do collect resin, the number of resin foragers depend on the needs of the colony (as discussed later in this chapter), but generally they are less than 1% of the total forager work force at any point in time. Resin collection is a very difficult and time consuming task to perform. After chewing pieces of resin from the plant, bees must transfer the sticky secretion from their mandibles to their hind legs before returning to the hive. Because of the sticky characteristics of resin, onceback in the hive, resin foragers need the assistance of other bees to remove the resin load from their legs, which may take up to 30 minutes (Nakamura and Seeley, 2006). The bees will then carry the resin in their mandibles to the site in the hive where the resin will be deposited. Honey bees naturally nest in tree cavities where they coat the entire inner surface of the nest cavity surrounding the combs with a propolis envelope (Seeley and Morse, 1976). It was suggested by Seeley and Morse (1976) that the propolis envelope had various functions, including serving as an impermeable barrier to tree sap and environmental moisture, a solid surface for comb attachment, a physical barrier to outside invaders by sealing the holes and cracks of the nest cavity, and finally, an antimicrobial layer against natural occurring fungi and bacteria in the tree cavity. When nesting in a hollow tree cavity, honey bees prepare the new nest site by removing the soft, rotten wood from the nest walls and depositing propolis in the cracks and top surface to make it solid and smooth (Seeley and Morse, 1976). Beekeepers, particularly in the U.S., have selected against colonies that collect large amounts of propolis (Fearnley, 2001) because its stickiness makes opening and managing colonies in standard beekeeping equipment difficult. Importantly, honey bees do not construct a propolis envelope within standard beekeeping equipment because the inner walls of the wooden boxes are already solid and smooth, which apparently does not stimulate bees to deposit propolis on them. Instead, bees deposit propolis in dispersed cracks and crevices in manmade bee boxes, and not as a continuous envelope as they do within a tree cavity (reviewed in Simone-Finstrom and Spivak, 2010).Honey bees are very resilient insects; they have thrived in this world for 6-8 million years (Engel, 1999), relying only on their own natural defense mechanisms to survive. Although propolis has been used as a traditional and natural human medicine since biblical times (Simone-Finstrom and Spivak, 2010), the benefits of propolis for honey bee health were not appreciated until we began research on this topic in the last decade. Our research has shown that the presence of a propolis envelope enshrouding the nestarea is a fundamental component of honey bee colony health. The propolis envelope functions as an antimicrobial, or disinfectant layer around the nest, and thus as an external layer of the colony immune system. This chapter will summarize current research questions we have explored in the past few years, since the previous review (Simone-Finstrom and Spivak, 2010), including: 1) the seasonal benefits of a propolis envelope to colony health and individual honey bee immunity; 2) the therapeutic role the propolis envelope plays in bees' natural defense against brood diseases; and 3) how honey bees select and use plant resins as a form of self-medication.

Resin collection and propolis use by honey bees:

Honey bees use propolis in varying degrees, some species and races rely verylittle on the substance, while others use resins and propolis extensively (Butler, 1949;Crane, 1990; Page *et al*., 1995). In fact propolis can be replaced by wax in honey beecolonies (Meyer, 1956; Crane, 1990). Colonies of Apisdorsata, the giant honey bee, mayuse resin occasionally to strengthen the site of comb attachment on a branch, while A.cerana colonies are not thought to use resins at all (Seeley and Morse, 1976; Crane,1990). On the other hand, resins are thought to be essential to A. florea (the dwarf honeybee). To prevent ants from invading their exposed nests, A. florea places a ring of resinon the branches leading to a nest (Crane, 1990; Seeley et al., 1982). Very limitedinformation exists on the use of resins by these Asian species of honey bees.Use of resins by A. mellifera colonies is much more widespread. While there isconsiderable variation among colonies in resin collection and propolis use, all colonies doappear to use at least some (Seeley and Morse, 1976; Page et al., 1995; Manrique andSoares, 2002; M. Simone-Finstrom, pers. obs.). A feral colony nesting in a tree cavitycoats the entire inner walls with a thin (0.3 to 0.5 mm) layer of propolis forming what hasbeen termed a "propolis envelope" around the nest interior (Seeley and Morse, 1976; Fig.1.1). Propolis is continually added to the nest walls during colony development, and isfirst placed at areas prior to comb attachment, which not only creates a clean, smooth surface, but may also reinforce new comb (Seeley and Morse, 1976; Visscher, 1980).Both feral colonies in tree cavities and domesticated colonies in commercial hive boxes,generally use propolis for covering holes and crevices in the nest, and narrowing the hiveentrance (Huber, 1814; Haydak, 1953; Ghisalberti, 1979), which is evident from theorigin of the word propolis ("pro": in front of; "polis": the city). Utilizing propolis in thismanner is thought to function as a way for colonies to better maintain homeostasis of thenest environment. This could be a result of reducing microbial growth on hive walls,preventing uncontrolled airflow into the nest, and waterproofing walls against sap (iftree-cavity nesting) and external moisture, in addition to creating some protection againstinvaders (Seeley and Morse, 1976; Ghisalberti, 1979; reviewed in Visscher, 1980).Because of the range of uses for propolis, it has been noted that propolis isessential to honey bees, particularly those in the wild (Haydak, 1953; Hoyt, 1965).However, domesticating bees has resulted in a reduction of propolis collection acrossraces (Fearnley, 2001), likely because its use by bees often makes opening hives moredifficult for beekeepers. Hoyt (1965) said that propolis "is the bane of a beekeeper'sexistence", so it is no surprise that apiculturists have selected lines that happened toproduce less propolis.

Seasonal benefits of propolis to bee immunity and colony health under natural field conditions:

A honey bee colony can be considered a superorganism, a group of related individuals living together in a nest with the ability to perform collective foraging, thermoregulatory and defensive behaviors. When collective behavioral mechanisms are used to defend the colony against parasites and pathogens, they are called mechanisms of social immunity (Cremer *et al*., 2007). Examples of social immunity in honey bees include hygienic behavior (the ability of adult bees to detect and quickly remove diseased and mite infested brood from the nest, limiting pathogen and parasite transmission; reviewed in Evans and Spivak, 2010), grooming (removal of the parasitic Varroa mite from a nestmate's body; Boecking and Spivak, 1999) and foraging for resins to form a propolis envelope inside the nest (Simone *et al*., 2009; Simone-Finstrom and Spivak, 2012).The benefits of the propolis envelope to honey bee health were first investigated in the lab at the University of

Minnesota by coating the inside of small managed hives with a propolis extract (solution of 13% propolis in 70% ethanol) with a paintbrush, and allowing bees to be exposed to this propolis-enriched environment for 7 days (Simone et al., 2009). After one week, 7-day old bees had lower immune system activation and lowerbacterial loads in and on their bodies compared to same-age bees in hives without the propolis-extract coating (Simone et al., 2009). These initial findings told us that bees in hives with the propolis envelope did not have to expend as much energy turning on (activating) their immune system to fight off microbes, presumably because there were fewer microbes in the nest. When the immune system of bees, or any animal, is activated it comes with a physiological cost such as reduced survival (Moret and Schmid-Hempel, 2000). In fact, the immune system is the most costly physiological system to maintain (Evans and Pettis, 2005; Schmid-Hempel, 2005). When the immune system does not need to be highly activated, as when there is a propolis envelope in the nest cavity, bees are able to allocate their energy to perform vital tasks (e.g. foraging, rearing brood) and to store protein in their bodies.Following up on Simone-Finstrom's Ph.D. research on the short-term benefits of the propolis-extract coating inside the bee hive, we were curious to know the long-term benefits of a propolis envelope that was naturally deposited by the bees. Recent research from Brazil showed that Africanized bee colonies that collect high amount of propolis, had greater brood viability, longer worker lifespan, higher honey production, more rapid hygienic behavior and larger pollen stores, compared to colonies that collect low amount of propolis (Nicodemo*et al*., 2013; Nicodemo*et al*., 2014).

Significance to bee health

Propolis is highly regarded for its medicinal properties for humans, especially inEastern Europe, South America, and Asia. The antimicrobial properties of propolisagainst human pathogens have been known since antiquity (see Ghisalberti, 1979). Anumber of studies have presented evidence that propolis has strong hepatoprotective,antitumor, antioxidative, antimicrobial and anti-inflammatory properties (for recentreviews see Banskota et al., 2001; Sforcin, 2007; Viuda-Martos*et al*., 2008). Curiously,few studies have examined the antimicrobial properties of propolis against bee pathogensor on honey bee immune responses. Since much of the background on biological activityofpropolis involves using propolis or components of propolis as treatments of disease,there has been a logical transition into studying propolis as a treatment to use in honeybee colonies (i.e. Samšinákov*áet al*., 1977; Garedew*et al*., 2004; Antúnez*et al*., 2008).However, there should also be a combined focus on the natural function of propolis,specifically determining if its presence in a honey bee hive either directly or indirectlyaffects pathogen and parasite loads. There is some evidence that it may both serve as anatural mechanism of disease resistance and have the potential to be further applied as anin-hive treatment.Here we describe completed research on the potential significance ofpropolis for bee health, and then discuss the future direction of this work.

Other large parasites and pests:

Honey bee colonies also must defend themselves against a number of largerparasites and pests. Two studies have examined the effectiveness of propolis extractsagainst the greater wax moth, an opportunistic parasite that mainly affects weakenedhives (Johnson *et al*., 1994; Garedew*et al*., 2004). In laboratory experiments similar tothose conducted with Varroa, propolis extracts caused larval mortality and reducedmetabolic rates of wax moth larvae and adults (Garedew*et al*., 2004). The implicationhere is that contact or possibly volatile emissions from propolis may reduce the ability ofthe moths to effectively reproduce and develop within a hive.With respect to other large invaders, Cape honeybees, A. m. capensis, have beenobserved encapsulating the parasitic small hive beetle, Aethinatumida, in “propolisprisons” which serves to prevent the beetles from successfully reproducing (Neumann *etal*., 2001). The European honey bee, *A. mellifera*, will also embalm other intruders thatare presumably too large to remove from the nest after being killed; Hoyt (1965)observed a mouse encased in propolis and suggested that the bees covered it in propolisto prevent odor and decay from affecting the rest of the hive (Fig. 1.2). Colonies ofA.dorsata have also been noted to coat foreign objects in propolis (Seeley and Morse,1976), as have the stingless bee Trigonacarbonaria that "mummify" beetle parasitesalive using a mixture of wax, plant resins and mud (also known as batumen; Greco *et al*.,2010). It may be that this behavior of embalming predators or parasites may be arelatively widespread phenomenon among the social bees. Particularly with respect tothis entombment behavior, the use of propolis by bees can be described analogously toindividual immune function. If we consider a honey bee colony as one entity or"superorganism", then this behavior would be equivalent to cellular encapsulation offoreign microbes or parasites seen at the individual level (see Cremer and Sixt, 2009).The propolis envelope itself, also fits into this analogy as it is a type of mechanical barrier to both reduce parasites from entering the nest (or superorganism) and potentiallyprevents parasites and microbes from developing once inside (i.e. Simone *et al*., 2009).

Future studies on bee health:

There have been a number of studies on the eff ectiveness of propolis against bacterial pathogens. Further studies should be con-ducted with respect to propolis against hive diseases both alone and in combination with other disease resistance mechanisms (i.e. hygienic behavior) to better determine how valuable propolis could be as a direct treatment. In Europe, there are currently plans to study how propolis may be used against bee pathogens and parasites as a form of treatment (see Moritz *et al*., 2010). Research at the University of Minnesota currently underway has a similar, but more specific focus.Propolis extracts in general have been shown to be active against some human viruses in vitro (i.e., HIV-1, Gekker*et al*., 2005), and the results of the work on honey bee viruses could have implications for human health by identifying possible compounds for further study.It is possible that the antimicrobial properties of materials used and stored in combs (e.g. royal jelly, honey) are enhanced by the addition of propolis (Visscher, 1980; Tautz, 2008). In particular, the modes of action of propolis against microbes and parasites are currently unknown and could be due to contact (e.g. Garedew*et al*., 2002) and/or volatile emissions (e.g. Messer, 1985). The two modes are not necessarily mutually exclusive and could have varying eff ects depending on the organism, and must be considered when investigating the use of propolis both as a colony treatment and its natural effectiveness in the hive.

Self medication:

In light of all of this information, one obvious question concerns the idea of "selfmedication."Resin collection may be constitutive (i.e., collected regardless of physiological demand or pathogen level) or inducible (i.e., a conditional response to infection; Schmid-Hempel and Ebert, 2003). If it is inducible, it might be considered a form of self-medication, defined as the "defense against [pathogens and] parasites by one species using substances produced by another species" (Clayton and Wolfe, 1993). There are number of vertebrates that self-medicate by ingesting, absorbing, topically applying or living in proximity to plants with medicinal compounds (reviewed in Clayton and Wolfe, 1993; Lozano, 1998). Examples of self-medication in the insect literature, particularly with respect to social insects are less common. When F. paralugubrisantcolonies were challenged with the fungal pathogen Metarhiziumanisopliae, they did not respond by increasing the rate or quantity of resin collection, and the authors concluded that the use of resin by this species was a constitutive rather than inducible response, and therefore not an example of self-medication (Castella*et al*., 2008b). Honey bee colonies infected with diseases or parasitic mites do not appear to respond by collecting more resin (M. Simone-Finstrom, M. Spivak, pers. obs.) but studies to quantify resin collection after pathogen challenge are ongoing (see Chapter 4). The trade-off between the energetic costs to individual bees of collecting resin may have been offset by the antimicrobial properties of the resins which benefited the individuals' immune systems and increased colony fitness, leading to continued selection for resin collection regardless of pathogen or parasite levels.

Sources of resin and the process of resin collection:

In tropical climates honey bees mostly collect resins from Clusia minor andClusiarosea flowers and from alecrim plants (e.g. Baccharisdracuncufolia), which issimilar to other tropical bee species (Pereira *et al*., 2003; Salatino*et al*. 2005). Recently aleguminous species (Dalbergia sp.) has also been identified as a common source intropical regions (i.e. Silva *et al*., 2008). In temperate climates poplar trees (Populus sp.)appear to be the primary source for resins (Popravko and Sokolov, 1980; Nagy *et al*.,1986; Greenaway *et al*., 1987; Bankova*et al*., 1992; 2006; Markham *et al*., 1996; Salatino*et al*., 2005). However, it is clear that other trees, like pine, birch, elm, alder, beech andhorse chestnut species, are adequate resin sources for temperate honey bees, particularlywhen poplar species are unavailable (Alfonsus, 1933; Ghisalberti, 1979; Crane, 1990).Additionally, honey bees in Uganda appear to forage for resins selectively on Alnussp.and can actually defoliate these trees; whether there are other possible sources in theregion remains unclear (Nyeko*et al*., 2002).Honey bees will forage for resins from droplets appearing on the bark of thetrunks or limbs of trees (Alfonsus, 1933), from the surfaces of some fruits (i.e.Macarangatanarius; Kumazawa*et al*. 2008), or more typically on the vegetative apices(buds, leaf primordia and young leaves). The bees must extract the resins from thetrichomes and ducts by fragmenting these early leaves using their mandibles (Meyer,1956; Nyeko*et al*., 2002; Teixeira *et al*., 2005). Resin foragers have shown a preferencefor young leaves and vegetative buds over more expanded leaves (Park *et al*., 2004).The cues that honey bees rely on to find resinous plant sources are currentlyunknown. Huber (1814) placed a bunch of poplar branches “that had very large budscoated both on the outside and inside with a viscous, reddish and odoriferous sap” infront of his honey bee colonies and observed bees collecting resins within 15 minutes. Itis clear that foragers select specific sources, and rely on currently unknown cues. Honeybees have been observed probing the apex of one plant with their antennae then movingto another one, probing it and subsequently collecting resin from it (Teixeira *et al.*, 2005).The same study also provided evidence that the resin foragers preferred female versusmaleBaccharisdracuncufolia as resin sources. The young leaves and buds have a similarchemical composition that changes as the leaves become more expanded (Park *et al*.,2004), which implies that there may be a chemical cue released by the resin source thatthe foragers are able to detect.Once the bees find the resin source, they then have to collect it. Huber (1814),Haydak (1953), and Meyer (1956) have described this process in great detail. There arefour basic steps (taken from Meyer, 1956) that a resin forager follows to pack hercorbicula: (1) Break off a particle of propolis with the mandibles; (2) work it with themandibles and take it with the forelegs; (3) transfer it from the forelegs to the middle leg;(4) transfer it from the middle leg to the corbicula on the same side. This sequence isrepeated until there is a full resin load on both corbicula. No corbiculatebees can collect resin and pollen during a single foraging trip because of this behavior(Armbruster, 1984; Roubik, 1989). After completing the four steps, bees have beenobserved flying around for a few seconds above the resin source, then landing again toadd more to each corbicula (Alfonsus, 1933; Haydak, 1953). The purpose of these flightsis unknown but may be used to assess the weight of the current corbicular load. Theprocess of obtaining a full corbicular load of resin has been noted to take about sevenminutes (Teixeira *et al*., 2005; Kumazawa*et al*., 2008), but can take from 15 min to anhour depending on the weather (Haydak, 1953).Once the bee has a full load, she returns to her colony to unload the resin from hercorbiculae. The unloading process typically takes approximately 15 minutes, but canextend from one to seven hours or even overnight (Alfonsus, 1933; Haydak, 1953;Ratnieks and Anderson, 1999; Nakamura and Seeley, 2006). A resin forager cannotunload her corbiculae herself, but rather must rely on her nestmates to take the resins offof her. Once the resin forager returns with a full load, she will go to a site within the hivewhere propolis is needed, where she waits until other bees, known as cementing bees,bite off chunks of resin from her corbiculae (Betts, 1921; Alfonsus, 1933; Haydak, 1953;Meyer, 1956; von Frisch, 1993; Nakamura and Seeley, 2006). Cementing beesimmediately attach the resin to a site along the hive wall. The cementing bee thensmoothes the resin, now officially propolis, with her mandibles in a manner that is similarto that of wax construction (Alfonsus, 1933; Nakamura and Seeley, 2006). The resinsmay also be placed in a storage area where bees can grab chunks of propolis to later placein comb cells or other areas (Huber, 1814; Haydak, 1953; Fearnley, 2001; Tautz, 2008).Many of the few resin foragers in a colony will perform cementing behavior, but not allcementing bees will forage for resins (Huber, 1814; Meyer, 1956; Nakamura and Seeley,2006). Meyer (1956) found that forager-aged bees with atrophied wax glands do most ofthe cementing work. Recent evidence from Nakamura and Seeley (2006), however,indicated that the bees they observed using resin in the nest performed these behaviorsprior to foraging. This suggests that cementing and other in-hive resin activities areperformed by the middle-aged bees that typically perform nest construction tasks inaddition to those bees foraging for resins.During the cementing process, the resins do not appear to be chemically modified.While there is some evidence that the general chemical profiles of resins collecteddirectly from a forager and in-hive collected propolis can vary slightly from the leaf budsof the plant source (i.e. Ghisalberti, 1979; Peev*et al*., 2009), it is likely that somevariation could occur to due volatilization of some chemicals during the course of thereturn foraging trip. In addition, propolis sampled from a single colony likely contains anamalgam of various sources at least to some degree in addition to wax and is essentially aconcentration of some of the compounds collected directly from the plants. Thus, somecompounds would be expected to be more or less represented in propolis samples, but thegeneral chemistry would remain similar as has been found (i.e. Greenaway *et al*., 1990;Park*et al*., 2004; Teixeira *et al*., 2005; Vardar-Ünlü*et al*., 2008). For other bee species,however, there is some suggestive evidence that bees add secretions to the resins.Workers of the stingless bee Plebeiaemerina reach maximum development of the headand intramandibular glands during the age of most frequent resin handling, which may beutilized to maintain the viscosity of resins during use (dos Santos *et al.*, 2009). How thismay change the chemical properties of the resins has yet to be investigated.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the districts of Bangladesh like Sirajgonj, Gazipur and Satkhira (Sundarban). This experiment was conducted on the collection of propolis and to know the effect on the bee health.This study was conducted during Nov 2015 to 30 May 2016 .

3.1 Experimental site

The present study on the propolis collection efficiency of *Apismellifera* L. was done is Sirajgonj, Gazipur and Satkhira districts. Three upazilla i.e. Ullahpara (Site 1), Shahzadpur (Site 2) and Tarash (Site 3) were selected in Sirajgonj for the study. Similarly GazipurSadar (Site 1), Kapasia (Site 2) and Kaligonj (Site 3) upazilla were selected in Gazipur districts. Moreover, Munshigonj (Site 1), Kaligonj (Site 2) and Tala (Site 3) upazilla were selected in sundarban areas of Satkhira district. The experimental duration was 15 November 2015 to 30 May 2016. Peak mustard flower blooming period, litchi blooming period and mangrove plants blooming period were selected for data recording. Data collection regarding the predetermined parameters and the analysis of data was performed to measures the efficiency of honey bee. The materials required and the methodology of the application of treatments and determining various parameters are described under the following sub-headings:

3.2 Experimental materials

Traditional single wooden box with seven framed bee poly hivesuper with proplis mesh. Traditional Gunny bag materials and poly proplis traps were used on the top of the frame under the cover of bee boxes.

Honey bee (*Apismellifera* L.) colonies:

To study the propolis collection efficiency of honey bee, *A. mellifera* L. colonies, 10 uniform hives from each of 2 apiaries of each site of the same species were selected. Each hive or colony is consisting of 7 frames (2 brood frames+ 3 occupied/2built frames) and a feeder frame. All the frames were considered for data collection.

Design and layout of the Experiment:

The amount of propolis hoarded by beecolonies was inferred from the amount ofpropolis loads which were collected bymeans trapswith a 5.00 mm mesh perforated plate.Propolis was collected weekly. Raw propolis were processed by standard method and dried and weight was taken. Paired plot techniques were used to compare the yield of traditional and polyhive boxes. Total 20 hives were selected for paired test 10 traditional and 10 polyhivetype. For health observation symptoms were observed in 20 bee hives in each treatments.

Statistical analysis:

The results were analyzed statistically using Excel software. Arithmetic means and standard deviations were calculated. The differences were tested for significance by means of Duncan’s multiple range test.


Fig: Propolis containing polythene and gunny bag


Fig:Extracted Propolis

Fig: Propolis extraction procedure

CHAPTER IV

RESULTS AND DISCUSSION

Figure 1 illustrates the comparison of average propolis yield per hive by using Sirajganj traditional hive and Sirajganj super hive in 3 sites of Sirajganj District. It shows that, there is no propolis yield in all three sites by using Sirajganj Traditional where using Sirajganj Super gives varying yields respective of site. From site 1, 16.83 g propolis has been obtained from per hive. Moreover, from site 2 and site 3, 16.5 g and 18.33 g propolis obtained per hive respectively. However, it is clear that the highest (18.33g) propolis is obtained from site 3 and the lowest (16.5g) propolis from site 2.

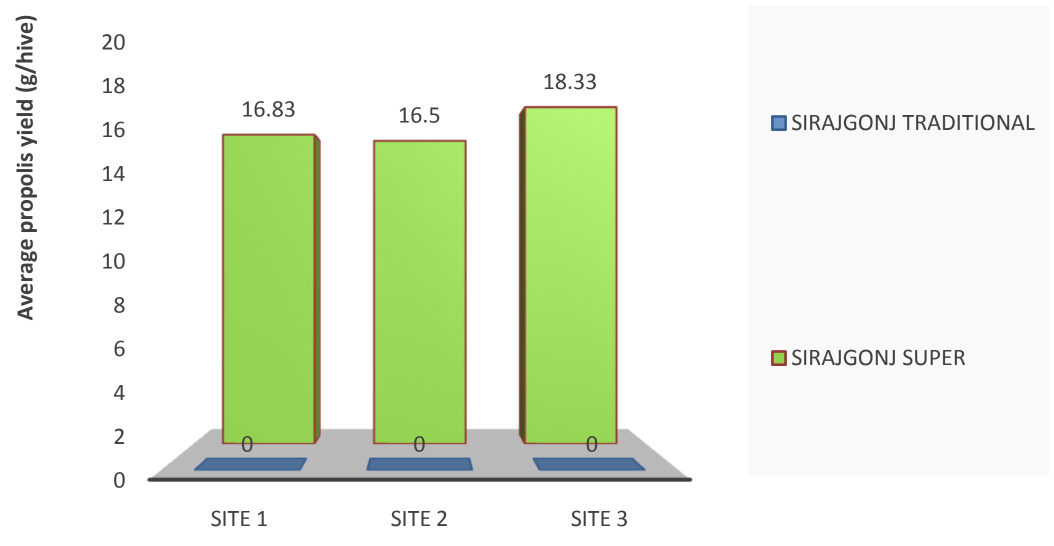

Fig. 1. Propolis yield in Sirajgonj district

It can be concluded that by using super hives propolis yield can be gained tremendously where traditional hives yielded no propolis.

In case of Bee health presence of propolis and box type reduces diseases infection and pests infestation in different observed hives in Sirajgonj.

In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed diseases EFB and Nosema positive whereas, in the modern polyhive bee boxes with propolis traps showed only Nosema positive (Table. 1)

Table 1: Propolisvs diseases and pests present in the bee hive in Sirajgonj district

Propolis trap type	Diseases*						Pests**			
	EFB	AFB	SV	CB	DWV	N	V	T	A	SHB
Traditional (n=20)	+	-	-	-	-	+	+	+	+	-
Polyhive super (n=20)	-	-	-	-	-	+	-	-	-	-

Diseases*: EFB=European Foul Brood, AFB= American Foul Brood, SV= Sac Brood, CB= Chalk Brood, DWV= Deformed Winged Virus, N= Nosema disease

Pests**: V= Varroa mite, T= Tropilaelaps mite, A= Acarapis mite, SHB= Small hive beetle

(+) = Present and (-) =Absent

In case of pests attack presence of propolis and box type reduces infestation in different observed hives in Sirajgonj. In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed Varroa, Tropilaelaps and Acarapis mite positive and Small hive beetle negative whereas, in the modern polyhive bee boxes with propolis traps showed no pests infestation symptom.

The following figure 2 showed the comparison of average propolis yield per hive by using traditional hive and super hive in 3 sites of Gazipur District. It showed that, there is no propolis yield in all three sites by using traditional where using Super gives varying yields respective of site. From site 1, 24.08 g propolis was obtained from per hive. Moreover, from site 2 and site 3, 26.42 g and 25.42 g propolis were obtained per hive respectively. However it is clear that the highest (26.42 g) propolis is obtained from site 2 and the lowest (24.08 g) propolis from site 1.

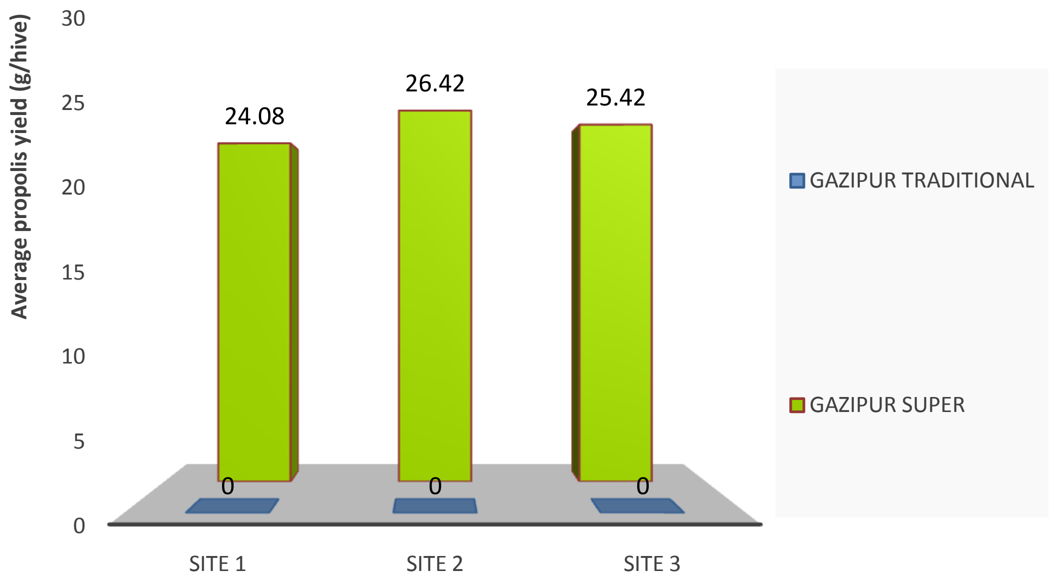

Fig. 2.Propolis yield in Gazipur district

From this figure it is concluded that in the traditional hive propolis could not be obtained by farmers. On the other hand from poly hive super propolis could be obtained by beekeepers.

In case of Bee health presence of propolis and box type reduces diseases infection and pests infestation in different observed hives in Gazipur.

In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed diseases only Nosema positive whereas, in the modern polyhive bee boxes with propolis traps showed no symptoms of diseases (Table. 2)

Table 2: Propolisvs diseases and pests present in the bee hive in Gazipur district

Propolis trap type	Diseases*						Pests**			
	EFB	AFB	SV	CB	DWV	N	V	T	A	SHB
Traditional (n=20)	+	-	-	-	-	+	+	+	+	+
Polyhive super (n=20)	-	-	-	-	-	-	-	-	-	+

Diseases*: EFB=European Foul Brood, AFB= American Foul Brood, SV= Sac Brood, CB= Chalk Brood, DWV= Deformed Winged Virus, N= Nosema disease

Pests**: V= Varroa mite, T= Tropilaelaps mite, A= Acarapis mite, SHB= Small hive beetle

(+) = Present and (-) =Absent

In case of pests attack presence of propolis and box type reduces infestation in different observed hives in Gazipur district. In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed Varroa, Tropilaelaps and Acarapis mite and Small hive beetle positive whereas, in the modern polyhive bee boxes with propolis traps showed Small hive beetle positive pests infestation symptom.

The following figure 3 showed the comparison of average propolis yield per hive by using traditional hive and super hive in 3 sites of Satkhira district. It showed that, there is no propolis yield in all three sites by using traditional where using Super gives varying yields respective of site. From site 1, 24.50 g propolis was obtained from per hive. Moreover, from site 2 and site 3, 27.50 g and 26.08 g propolis were obtained per hive respectively. However it is clear that the highest (27.50 g) propolis is obtained from site 2 and the lowest (24.50 g) propolis from site 1.

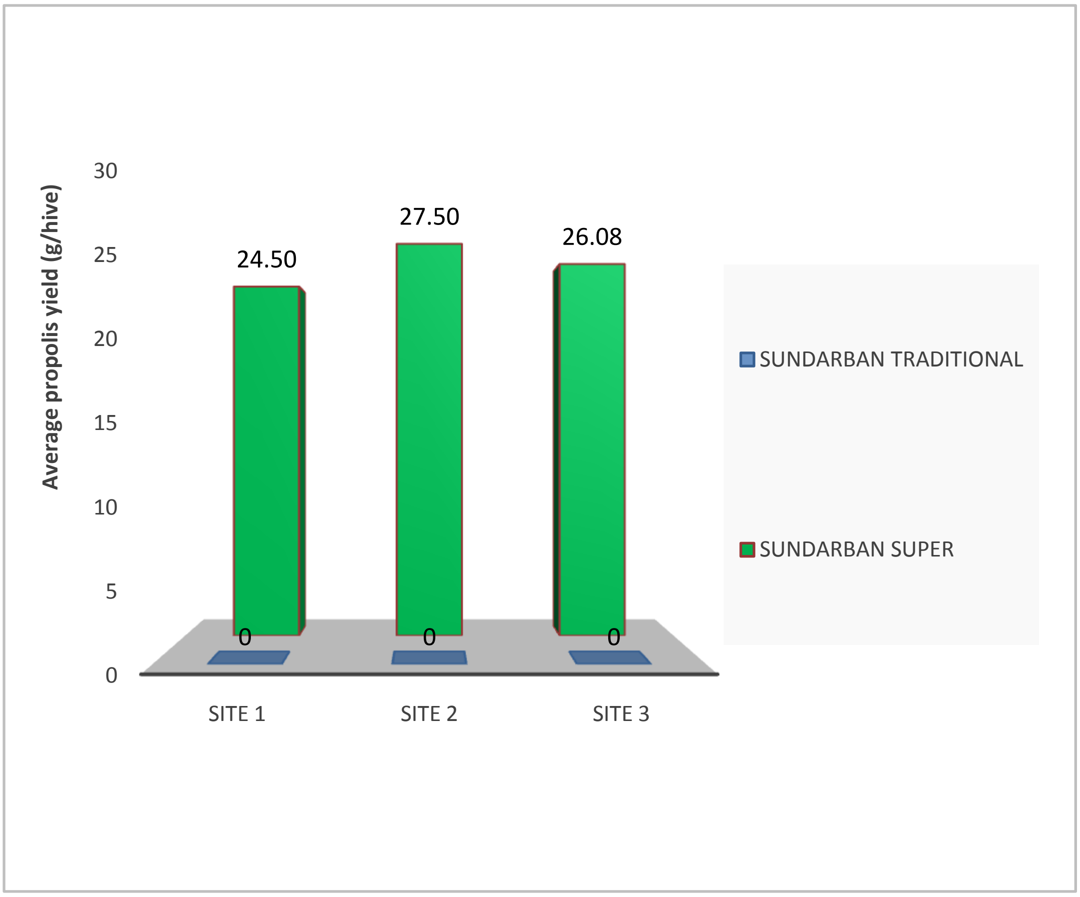

Fig. 3.Propolis yield in Shatkhira district

From this figure it is concluded that in the traditional hive propolis could not be obtained by farmers. On the other hand from poly hive super propolis could be obtained by beekeepers.

In case of Bee health presence of propolis and box type reduces diseases infection and pests infestation in different observed hives in Satkhira.

In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed diseases EFB and Nosema diseases positive whereas, in the modern polyhive bee boxes with propolis traps showed only EFB symptoms of diseases (Table. 3)

Table 3: Propolisvs diseases and pests present in the bee hive in Gazipur district

Propolis trap type	Diseases*						Pests**			
	EFB	AFB	SV	CB	DWV	N	V	T	A	SHB
Traditional (n=20)	+	-	-	-	-	+	+	+	+	-
Polyhive super (n=20)	+	-	-	-	-	-	-	-	+	-

Diseases*: EFB=European Foul Brood, AFB= American Foul Brood, SV= Sac Brood, CB= Chalk Brood, DWV= Deformed Winged Virus, N= Nosema disease

Pests**: V= Varroa mite, T= Tropilaelaps mite, A= Acarapis mite, SHB= Small hive beetle

(+) = Present and (-) =Absent

In case of pests attack presence of propolis box type reduces infestation in different observed hives in Satkhira district. In traditional hive and gunny bag using in the top bars inside the cover of bee boxes showed Varroa, Tropilaelaps and Acarapis mite positive, and Small hive beetle negative whereas, in the modern polyhive bee boxes with propolis traps showed Acarapis mite positive pests infestation symptom.

Figure 4 illustrates the comparison of average propolis yield using Traditional hive and super hive in 3 districts of Bangladesh. It shows that, there is no propolis yield in all the three districts by using Traditional hive whereas, using Super hives gives varying yields have been found. From Sirajganj district on an average 17.22g propolis was obtained from per hive. Moreover, from Gazipur and Shatkhira, 25.31g and 26.03g propolis have been collected per hive respectively.

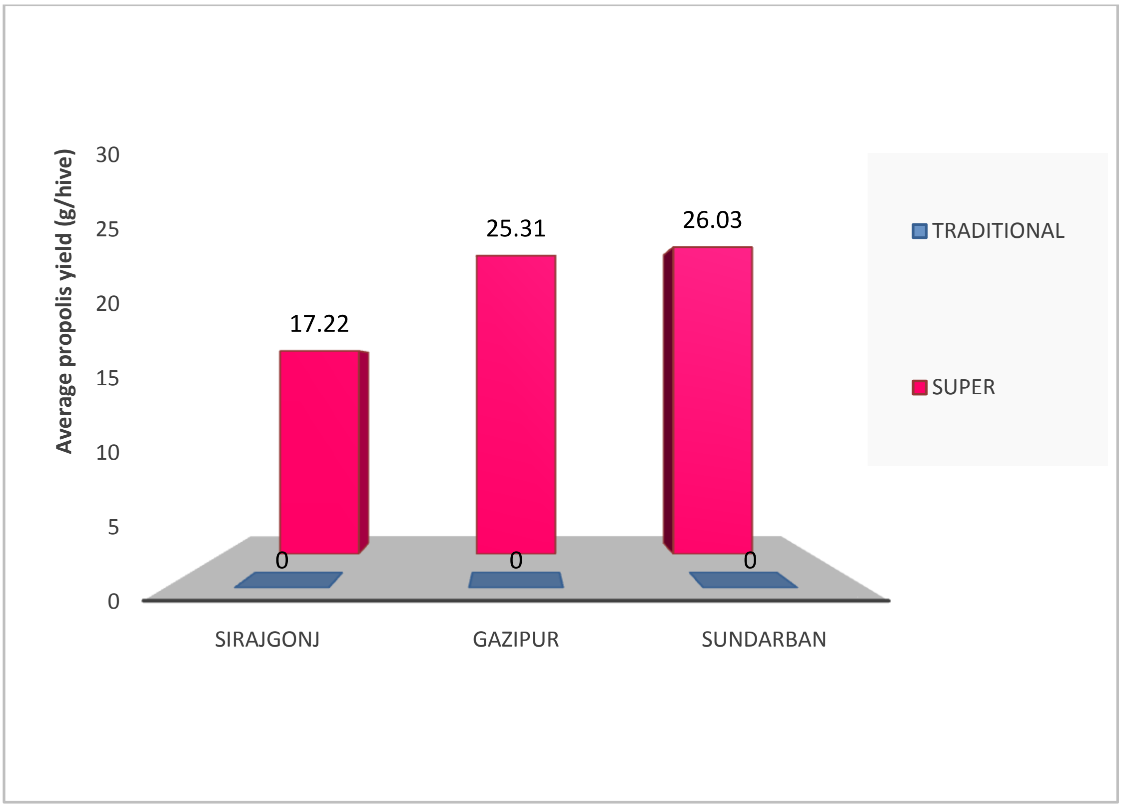

Fig. 4.Propolis yield per hive in different district

However, it is clear that the highest (25.31g) propolis was yielded from Sundarban area of Satkhira district and the lowest (17.22g) propolis from Sirajgonj district. It can be concluded that by using super hives propolis yield can be gained tremendously where traditional hives yield no propolis. It might be the availability of differest forests plants available in Sundarban areas of Bangladesh.

This figure 4 illustrates the scenario of total propolis yield by using traditional hive and super hive in 3 districts of Bangladesh. It shows that, there is no propolis yield in all three districts by using traditional hive where using Super hives gives varying yields have been found. From Sirajganj district, 310g propolis has been obtained from totally in Gazipur and Shatkhira, 455.50g and 468.50 g propolis have been collected all-out respectively. However it is clear that the highest propolis is achieved from Shatkhira (468.50g) and the lowest propolis from Sirajgonj (310.0 g).

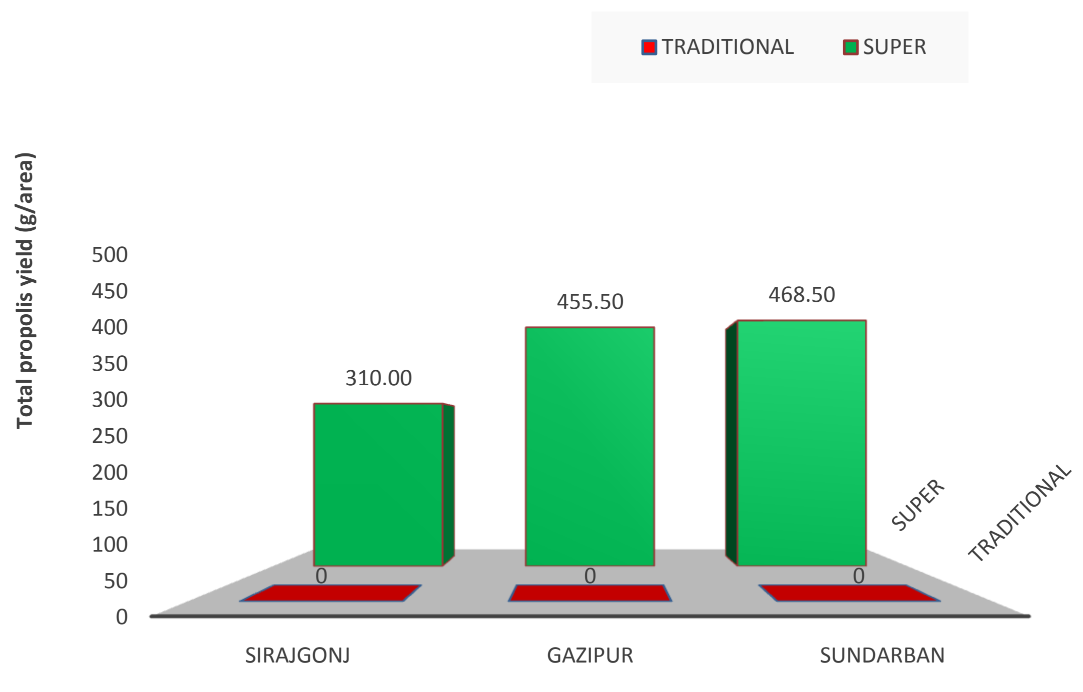

Fig. 5. Total propolis yield in different district

It can be concluded that by using super hives propolis yield can be gained tremendously where traditional hives yield no propolis.

CHAPTER V

Conclusion

The experiment was conducted at the field of Gazipur, Shundurban and sirajgonj, during the period from November 2015 to October 2016.In conclusion, the study provides new insights into the functional properties of propolis as acolony level defense mechanism and thereby further supports its substantial role for honeybee colony health. Traditional hives utilized by farmers are not good for propolis production and polyhive boxes with propolis mesh is good for propolis harvest. In each experimental site poly hive yielded the maximum amount of propolis in comparison to traditional hives. Again, propolis have effect on bee health. In each site of the experiment propolis contained hive showed minimum level of pests and diseases infestation in comparison to tradition hives.

CHAPTER VI

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