Ecological distribution of honeybee chalkbrood disease (*Ascosphaera Apis)* in Ethiopia

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Abstract

This study was conducted in Oromia, Amhara and Benishangul-gumuz regional states of Ethiopia. It was aimed to assess the infection and distribution rates of honeybee chalk brood disease (HCBD), analyse association of the disease with different bioclimatic zones and determine seasonal occurrence of the disease in Ethiopia. One- hundred and thirteen (113) peasant associations from 33 Woredas of the study regions were selected and 264 beekeepers were interviewed. A total of 1871 honeybee colonies from 133 apiaries were diagnosed externally and internally for HCBD. The infection rates of chalk brood disease in Amhara, Oromia and Beneshangul Gumuz Regional States were 37.12%, 19.89% and 17.93% respectively while distribution rates in same regions were 87.5%, 56.56%, and 33.33%. Similarly, the infection and distribution rates of HCBD were varied from zone to zones. High proportions of the respondents reported that HCBD occurred from September to November (43.6%) and from March to May (34.8%). There was significant honey yield variation between infected with HCBD and uninfected honeybee colonies ($P < 0.001$). Moist dega, moist weina dega and wet weina dega were identified to be the best suitable ecological zones for HCBD distribution. We concluded that there is high distribution of HBCD in all areas. Therefore; there is a need of avoiding transfer of honeybee products and by products from infected colonies to different places, awareness creation among beekeepers, strengthening colonies and design appropriate control strategies to combat the disease.

Key words: *Ascosphaera apis (A. apis*), Chalk brood, ecology, Ethiopia, Honeybee brood disease, bioclimaitic zones, seasonal occurrence

Introduction

Beekeeping in Ethiopia is a long-standing agricultural practice. Though beekeeping is practiced as a sideline activity, many of the rural farming com-

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munity generate substantial income from honey and beeswax selling and get benefit from the diverse use of honey. The current national average honey yield is estimated at 30 thousand tons per annum. This contributed 23.75% to the total Africa honey production and 2.48% to the total world production (MOARD, 2005). The current national beeswax yield is also estimated at 3 thousand tons per annum. Ethiopia is currently known to be one of the ten leading producers of honey and beeswax in the world (MOARD, 2005). Moreover, beekeeping has high contribution in enhancing food security, poverty reduction and food production through pollination of crops. This economically and ecologically important insect has been attacked by various pathogens (fungi, bacteria, parasite etc.). Fungi are commonly saprophytes of dead bees and combs. Some of the fungal species such as *Ascosphaera apis, Aspergillus spp*. and few others are considered to be honeybee fungal pathogen (Glinski and Buczek, 2003).

Honeybee chalk brood disease (HCBD) is a fungal disease principally attacking the brood of honeybees (*Apis mellifera L. Himenoptera: Apidae*) with ultimate death of infected larvae. It is caused by a fungus called *Ascosphaera apis.* At first the fluffy white mould covers dead larvae inside recently capped cells and later they dry and become black or white mummies. HCBD is reported to be most frequent in damp condition. The disease is widespread among honeybees in Europe and North America; infection rates in colonies of different areas in western Canada range from 20 %-39 % (Nelson *et al.,* 1977). Yakobson *et al*., 2003 reported that in Israel, chalkbrood was considered by the beekeepers to be the most important brood disease. These authors also indicated that in Germany levels of chalkbrood infection were generally low (1-15%) probably due to the favourable climatic conditions and nectar availability and the highest infection rates were observed in the hot and humid months between June and October (Yakobson *et al*., 2003). Although chalkbrood is aggravated by dump condition it is seldom found in New Zealand even in high rainfall district of West Coast of South Island during hot period (Bailey, 1959; Bailey, 1967; Bailey and Ball, 1991).

In Africa it was first reported from Tunisia (Heath, 1985).In the study conducted in Egypt to asses losses in honey yield due to chalkbrood disease in clover and chinus honey yields, it was found to be 18.41 % and 18.33 % respectively (Zaghlou *et al*., 2005). In Ethiopia chalkbrood disease was reported in the beginning of the year 2001. It was detected for the first time in the apiary of Holetta Bee Research Centre (HBRC) and its surroundings (Dessalegn, 2001, 2006). About 27 % of honeybee colonies in West Shewa, East Shewa, North Shewa and Arsi zones of Oromia Regional State were infected by HCBD

(HBRC, 2004). Currently reports claiming the existence of the disease are coming from all directions in the country indicating that HCBD of honeybee is becoming a threat to the development of beekeeping sector.

Therefore, to ensure sustainable beekeeping it is of paramount important to generate information on prevalence and infection rates, analysing association of the disease with different bio-climatic zones and seasonal distribution of honeybee chalkbrood disease in Ethiopia.

Materials and Methods

Study area

The study was conducted in Illuababor, Jimma, West shewa, Arsi and East Wollega zones of Oromia, South Wollo, West Gojam and North Gonder zones of Amhara and Asossa zone of Beneshangul Gumuz regional states from March 2004 to November 2006. The selected representative Woredas were from East Wollega (Diga, Leka Dulecha, Gutowayu, Sibu Sire), Jima (Goma, Mana, Gera, Kersa), Illababora (Metu, Bedele, Chora, Yayo Hurumu),West Shewa (Bako, Cheliya, Ambo), Arssi (Limubilbilo, Tiyo, Robe, Bokoji), South Wollo (Desie zuria, Kalo, Ambasel, Amahra Saynt), North Gonder (Adiareqey, Gonder zuria, Layarmacho, Chilga),West Gojam (Baherdar zuria, Bure, Mecha) and Asosa (Asosa, Maokomo, Bambasi). Purposive and stratified random sampling techniques were used to select the nine zones and 33 woredas (district) and peasant associations based on honey production potential, high number of honeybee colonies, accessibility and willingness of the beekeepers to participate in the study. Moreover, the study sites that were suspected to be entry areas for HCBD from outside of the country and major agro-ecological zones were represented. One hundred and thirteen (113) peasant associations from 33 Woredas of the study zones were also randomly selected. The selection at all level was performed with offices of MOARD and Keble's representatives. Geographical coordinate and altitude of the study areas were recorded using Garmin 4.5 hand- held Global Positioning System (GPS)

Survey, sampling and diagnoses of honeybee brood diseases

The sampling units were households keeping apiaries. A total of two hundred and sixty-four beekeepers (households) were randomly selected from the districts and interviewed using pre-structured questionnaire, which helped to assess the distribution of the disease, effects on general production or yield of honey and its seasonality. Each respondent was interviewed to estimate honey yield from infected and uninfected colonies at the same time since the majority of inspected colonies were found positive for HBCD.

Data were collected in single visit interviews during the main brooding seasons of the study areas. A total of 1871 honeybee colonies from 133 apiaries (1594 honeybee colonies from 111 apiaries in Oromia, 132 honeybee colonies from 16 apiaries in Amhara and 145 honeybee colonies from 6 apiaries in Beneshangul Gumuz regional states respectively) were diagnosed clinically by external and internal examination of the colonies (Hornitzky, 2001). External examination include observation of the colonies management, type of hives, dead brood/ mummies/ removed by worker bees in front of hives and in the entrances of honeybees. The internal examination was performed by opening the hives when suspected for chalkbrood disease and observe clinical evidences of the disease that are characterized by dead larvae in capped cells , dried/mummi-fied/cadaver reminiscent of a small piece of chalk, which become dark if fruit-ing bodies of fungi are formed (sporulating mummies) (Hornitzky, 2001).

The prevalence and infection rates of honeybee chalkbrood disease were analysed using descriptive statistics. T test was used to compare honey yield of infected and uninfected colonies.

Infection rate (%) = (Number of bee colonies that were found positive/ Total bee colonies examined) x 100,

Distribution rate (prevalence) (%) = (Number of study sites where the diseases were found/ Total numbers of sites where samples were collected) x 100

Ecology of CHB disease causative agent

The geographical position of localities where HCBD occurred was recorded using Garmin 4.5 hand- held Global Positioning System (GPS) to establish the ecology of the disease. Number of inspected colonies, existence of infection and infection rate of HCBD were presented along northing and easting data and used as input for BIOCLIM ecological niche analysis with DIVA-GIS software (Busby, 1991) and WORLDCLIM 30x30 arc-second global climate data (Hijmans *et al*., 2005).

Bioclimatic data selection

Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables. These are often used in ecological niche modelling (e.g., BIOCLIM, GARP). The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation); seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters).

BIOCLIM's definition of a quarter is any 13 consecutive weeks, (or any consecutive 3 months if running with a monthly time step) and is not necessarily aligned to any calendar quarters. For example, the driest quarter will be the 13 consecutive weeks that are drier than any other set of 13 consecutive weeks.

Bioclimatic variables are coded as follows: Annual Mean Temperature; Mean Diurnal Range; Isothermality; Temperature seasonality coefficient of variation; Maximum Temperature of Warmest Period; Minimum Temperature of Coldest Period; Temperature Annual Range (5-6); Mean Temperature of Wettest Quarter; Mean Temperature of Driest Quarter; Mean Temperature of Warmest Quarter; Mean Temperature of Coldest Quarter; Annual Precipitation; Precipitation of Wettest Period; Precipitation of Driest Period; Precipitation Seasonality coefficient of variation; Precipitation of Wettest Quarter; Precipitation of Driest Quarter; Precipitation of Warmest Quarter; Precipitation of Coldest Quarter.

This scheme follows that of ANUCLIM model, except that for temperature seasonality the standard deviation was used because a coefficient of variation does not make sense with temperatures between -1 and 1).

Generating the suitability prediction

When running BIOCLIM with the observation points as input, the input file needs to contain the independent variables used in the creation of the surface coefficient files. For BIOCLIM, the variables are usually the latitude (or Northing), longitude (or Easting) and elevation. The profile is a statistical summary of the bioclimatic parameters from each location and contains the following values for each bioclimatic parameter:

Not suitable,

0 to 2.5 percentile (low),

2.5 to 5 percentile (medium),

5 to 10 percentile (high),

10 to 20 percentile (very high),

20 to 34 percentile (excellent)

BIOCLIM computes the bioclimatic parameters for all of study sites, and then summarizes them, parameter by parameter to describe the climate that the causative agent HCB disease was found in.

Results

Infection and distribution rates of honeybee chalkbrood disease

In this study chalkbrood disease was found in all study regions (Oromia, Amhara and Beneshangul Gumuze Regional States) except dry bereha and moist bereha. The disease was detected in the majority of the study areas in these regional states (Table 1).

Table 1. Infection rates of CHB disease of honeybees in nine Zones of the study area

Region	Zone	No. Colony diagnosed	CHBD+ (%)
Oromia	E/Wollega	171	20 (11.7%)
	Jimma	499	127 (25.45%)
	Illu abbabor	418	81 (19.38%)
	W/Shewa	279	24 (8.6%)
	Arsii	227	65 (28.63%)
	South Wollo	25	16 (64%)
Amhara	N/Gonder	58	11 (18.97%)
	W/Gojam	49	22 (44.9%)
Beneshagul Gumuz	Assosa	145	26 (17.93%)
Grand Total		1871	392 (21%)

There was HBCD infection and distribution rates variation within and among the study regions. Infection and distribution of HCBD was found to be higher in Amhara Regional State compared to Oromia and Beneshangul Gumuze Regional States (Fig. 1 and Table 4). The infection rate in Amhara, Oromia and Beneshangul Gumuze Regional States was 37.12%, 19.89% and 17.93% respectively and distribution rate was 87.5%, 56.56%, and 33.33% in Amhara, Oromia and Beneshangul Gumuz, respectively (Fig. 1). Though there was no much infection rate variation between Oromia and Beneshangul Gumuze Regional States, the distribution rates of HCBD was varied in both regions (Table 4).

Figure 1. Chalk brood infection and distribution rates in Oromiya, Amahara and Beneshangul Gumuze Regional States

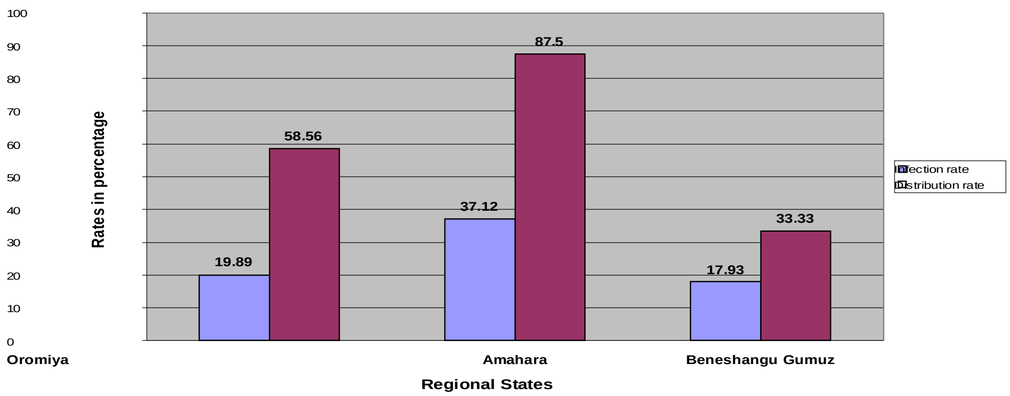

Figure1. Infection and distribution rates of CHB disease of honeybees in three regional states of Ethiopia

The infection rates of HCBD were varied from zone to zones in all regions. In Oromia the highest infection rate was found in Arsi (28.63%), followed by Jimma (25.45%) and Illuababor (19.38%) zones. The lowest infection rate was found in West Shewa (8.6%) and East Wollega (11.7%) zones (Table 1).

In Amahra the highest infection rate of HCBD was recorded in South Wollo zone (64%) followed by West Gojjam (44.9%) and North Gonder (18.97%) in the descending order (Table 1). Similarly the distribution rate was found to be much higher in West Gojjam (100 %) and North Gonder (80%) zones of Amhara Regional State while in Oromia the most affected zones are Jima (95.24) and Iluababora (64.7%) (Table 4).

Honeybee chalkbrood disease occurred during all seasons in study areas except June to August (Table 2). High proportions of the respondents reported that chalk brood disease occurred during September through November (43.6%)

and March through May (34.8%). These two seasons are times of honeybee brooding in Ethiopia and are conducive for *Ascosphaera apis* germination and sporulation in different parts of the study regions.

There was significant honey yield difference between infected and uninfected honeybee colonies by chalkbrood disease ($p < 0.001$). Chalkbrood disease affects honey yield negatively. In this study it is indicated the HCBD reduces the annual honey yield by 64 % (Table 3).

Table 2. Seasonal occurrences of chalk brood disease in the study regional states (%)

Region	Zone	No.	Season							
			September to November		December to February		March to May		June to August	
			Freq.	%	Freq.	%	Freq.	%	Freq.	%
Amhara	N. Gonder	12	5	41.7	2	16.7	5	41.6	0	0
	W. Gojjam	23	11	48	5	21.6	7	30.4	0	0
	S.Wello	17	5	29.4	7	41.2	5	29.4	0	0
Oromia	Jimma	43	20	46.5	14	32.5	9	21.0	0	0
	I/ Ababor	36	16	44.4	10	27.8	9	25.0	1	2.6
	E. Wellega	28	11	39.3	4	14.3	13	46.4	0	0
	W.Shewa	38	10	26.3	5	13.1	23	60.5	0	0.0
	Arsii	52	29	55.8	6	11.5	16	30.8	1	2.0
Beneshangul	Asosa	15	8	53.3	2	13.3	5	33.3	0	0
	Total	264	115	43.6	55	20.8	92	34.4	2	0.8

Table 3. Mean honey yield (Kg) comparison between chalkbrood disease infected and uninfected honeybee colonies in the study regional states

Status of honeybee colony	N	Mean	Sta. dev.	Minimum Honey yield Kg/colony	Maximum honey yield Kg/colony
Infected	264	2.43a	5.17	0.0	45
Uninfected	264	6.15b	11.10	0.3	80

Means with different letters are significantly different ($p < 0.0001$)

Table 4. Distribution rate (%) of honeybee chalkbrood disease in study Regional States

Region	Zone	HCBD distribution percentage
Amhara	N. Gonder	80.0
W. Gojam		100
S. Wollo		75
Oromiya	Jima	95.24
Oromiya	Ilubabor	64.7
	E. Wollega	25.0
	W. Shoa	40.0
	Arsi	45.45
Beneshangul Gumuz	Asosa	33.33

Ecology of CHBD

Bioclimatic predictions

A map of suitability was generated using the conditions described above by the 19-bioclimatic factors. The resulting map indicates the likelihood of presence of HCBD under the climatic conditions picked under each point of observation where HCBD was detected. Subsequently, the map was visually compared to the 19 individual climatic maps. Out of 19 climatic factors used in the model annual mean temperature, Isothermality, mean temperature of the wettest quarter, precipitation of wettest quarter, precipitation of warmest quarter and precipitation of coldest quarter seem to have explanatory power for the models result more than the rest. This observation is confirmed by finding quasi-iden-tical result when only six of the climatic factors used (Figure 2 and 4).

Comparison with Agro-climatic zones

Overlaying the raster data obtained from the Bioclim model on a raster of agro-climatic zones' classes can give us a picture of how the disease is likely to be distributed according to the Ethiopian climate zones (Figure 3).

It was found that dry alpine, dry bereha and moist bereha areas are not suitable for the occurrence of chalk brood disease at all. On the other hand a significant portion of the area within the very high and excellent suitability classes lies in the moist *dega*, moist *weina dega* and wet *weina dega* climatic zones, 9.1%, 10.4%, and 14.9% respectively.

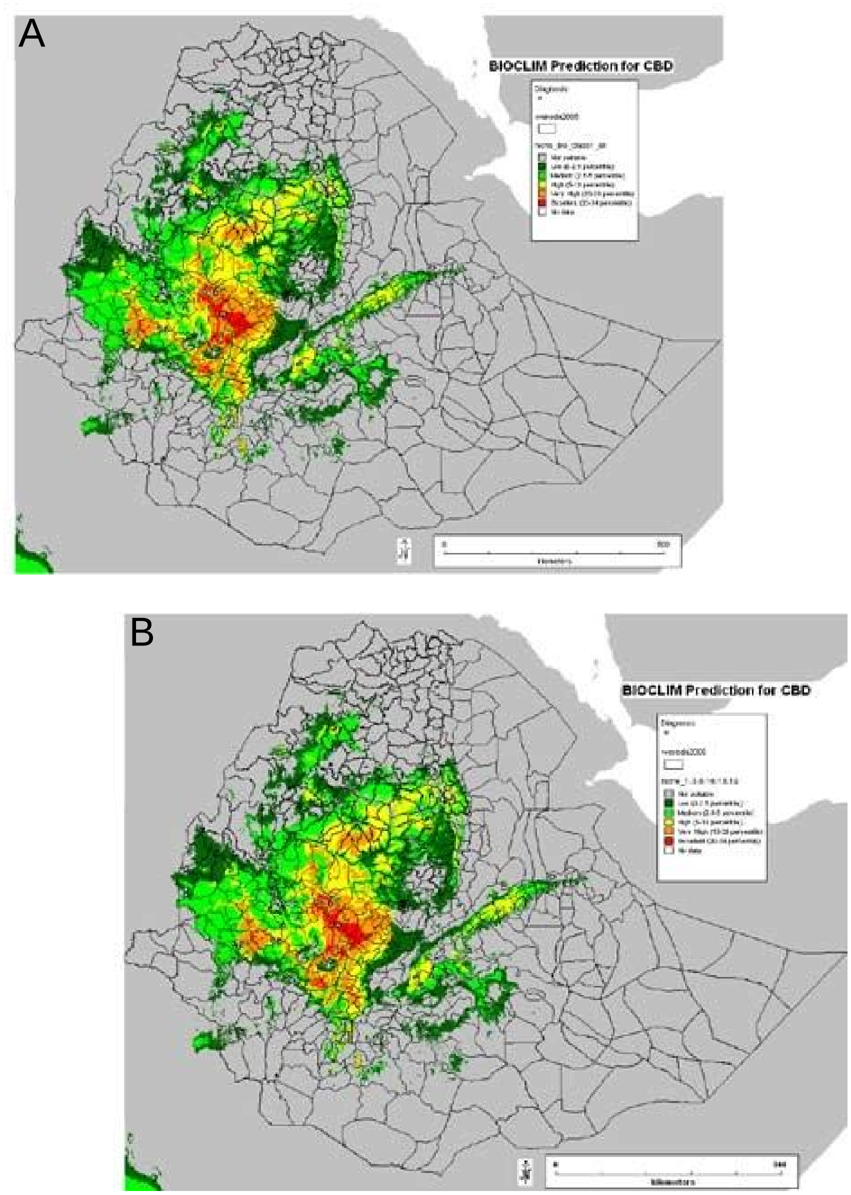

Figure 2. BIOCLIM model using all variables (A) and selected variables (B) for prediction of HBCD occurrence.

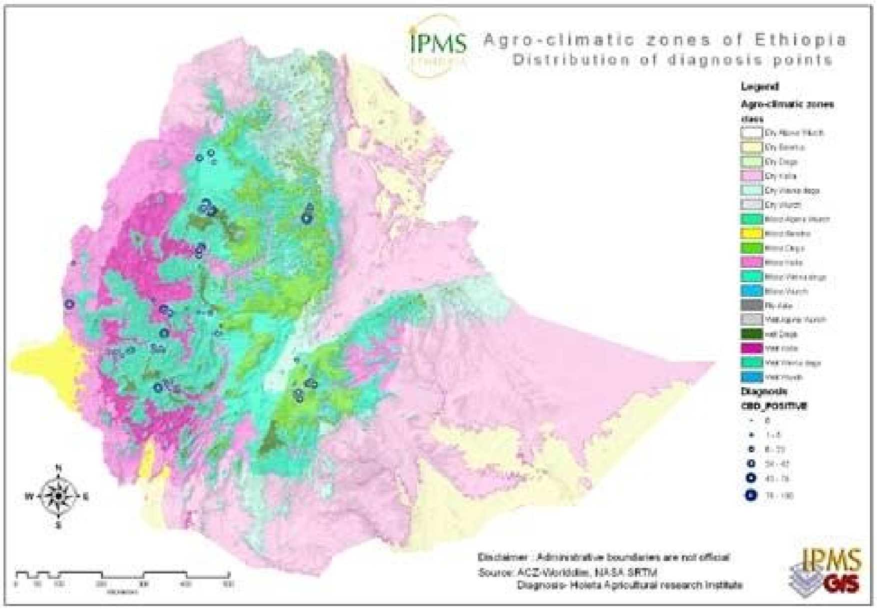

Figure 3. ACZ of Ethiopia- Distribution of observation sites with CBD positive percentages

Discussion

During the survey period attempts were made to inspect different brood diseases along with HCBD. However, chalk brood disease was the only brood disease detected in all parts of the study areas. The results of the present study clearly demonstrated that chalk brood disease was widely distributed in Amhara, Oromia and Benishangul Gumuz regional states of Ethiopia.

The infection rate and the distribution rate unequivocally indicated the seriousness of the disease. The infection rate observed in this study (17.93 % -37.12 % as summarized by regions) was similar to infection rate reported from western Canada where it ranged from 20% - 39% (Nelson et al., 1977). It was also observed that there was difference in rates of infection and distribution among the different zones of Oromia and Amhara. It was found that in those zones where bee production extension package has been practiced and where various beekeeping equipments were distributed had relatively higher distribution rate (for example West Gojam, (100%), Jima (95.24 %) of HCBD than

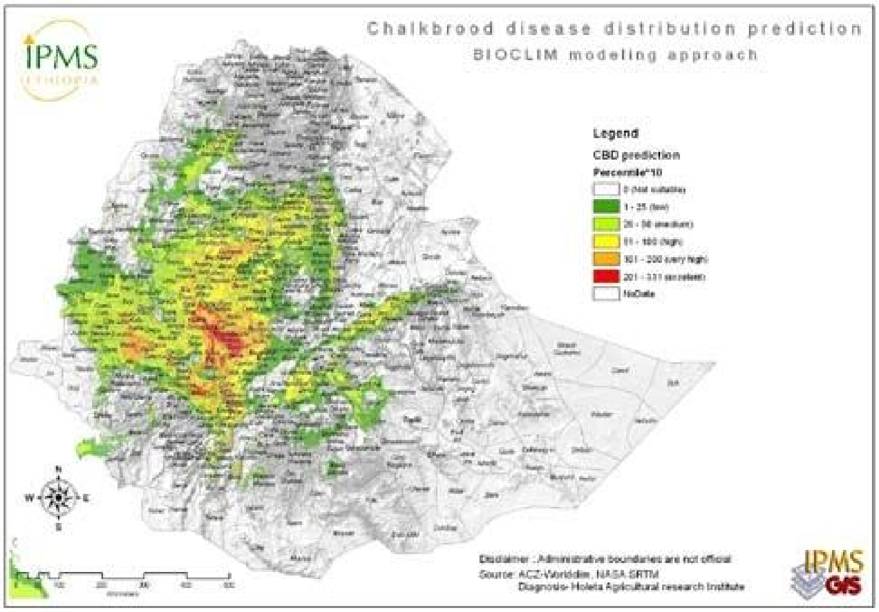

Figure 4: BIOCLIM model using only selected variables for prediction of HBCD occurrence

in those zones where relatively little intervention was done like Asosa and East Wolega (Table 4). This observation has probably led to the conclusion that the distribution of the causative agent of HCBD might be associated with delivery of contaminated apiary equipments. For instance, wax foundation sheet contaminated with spores of A. apis was shown to be a source of HCBD (Flores et al., 2005).

Based on the observations made, older hives, poor sanitation, negligence of the beekeepers to keep clean apiaries and lack of awareness about the disease among the beekeepers and other agricultural development workers found to be the prominent factors that might contribute for the high infection and wider distribution rate across all regions in the country. Seasons between September to November and from March to May were found to be peak seasons for occurrence of HCBD, which coincides with the brood rearing time of the year in the respective study sites. These months represent the cold, humid and hot seasons, which are conducive for multiplication of the causative agent of HCBD. Hence it agreed with the observation made by (Gilliam *et al*. 1977) who observed that heavy infestation in the USA through out the year even when average monthly temperatures were 29 °C.

A significant portion of the area within the very high and excellent suitability classes lies in the moist *dega*, moist *weina dega* and wet *weina dega* climatic zones, 9.1%, 10.4%, and 14.9% respectively. This finding agreed with the fact that HCBD is more prevalent in damp and cool conditions and aggravated when the brood is chilled (Dadant *et al*., 1975, Desalegn, 2006).

Conclusions

The significant prevalence and rapid spread of chalk brood disease and loss of honey production up to 64% due to the disease necessitates prompt attention from the relevant bodies for urgent implementation of appropriate control measures including setting up policy that could facilitate execution of monitoring activities. These may include control of movement of honeybee products, by-products and apiary equipments that could serve as a source of contamination and infection. In spite of the loss that the disease is incurring, there was lack of awareness about the importance of the disease among woreda beekeeping experts and the beekeepers themselves. The disease has not been recognized and all concerned bodies have taken no measures. Therfore, awareness creation is very important among all stakeholders.

So far there are no successful chemotherapeutic options for treatment or control of HCBD. Maintaining strong healthy colonies has been demonstrated to reduce the effects of chalkbrood disease elsewhere. Introduction of good management practices that could help to stop propagation of the pathogen should be in place. Hence, it is recommended to investigate practicable and effective options to control CHBD under Ethiopian condition. These may include investigation of potent chemicals that could help to disinfect apiaries and beekeeping equipments and to treat infected colonies and exploration of improved management practices that could help to reduce incidence and spread of HCBD.

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References

BAILEY, L. 1959: Infectious diseases of the honey bee. Rep. Rothamst. exp. Sta. 204-215.

Bailey, L. 1967. The effect of temperature on the pathogenicity of the fungus Ascosphaera apis, for larvae of the honey bee, Apis mellifera. In Proceedings of the International Colloquium on Insect Pathology and Microbial Control (P.A. vab der Laan, Ed), North-Holland, Amsterdam. pp. 162-167.

Bailey L; Ball BV. 1991. Honey Bee Pathology. Academic Press, London, p53-63; p154-158.

Busby, J.R., 1991. Biochlim – A bioclimatic analysis and prediction system. In: Nature conservation: Cost Effective Biological Surveys and data Analysis (Eds: Magules, C. R. and Austin, M.P), Melbourne: CSIRO. Pp. 64-68.

Dadant, 1975. Chalk brood, (In: The hive and the honeybee. Dadant Publication, USA)

Desalegn, B., 2001. Annual Report. Holleta Bee Research Center, (Memo).

Desalegn B., 2006. The occurrence of chalk brood (Ascoshaera apis): A honeybee (A. mellifera L.) Disease in West Shoa, Ethiopia. Ethiopian Journal of Animal Production, 6(1), 1-8.

Flores, J.M., Spivak M. and Gutierrez I., 2005. Spores of Ascosphaera apis contaminated in wax foundation can infect honeybee brood. Veterinary Microbiology. 15, 141-144.

Glinski, Z. and Buczek, K. 2003. Response of apoidae to fungal infection. APIACTA. 38: 183-189.

Gilliam, M., Taber S. and Rose J. B. 1977. Research on chalk brood diseases ofhoney-bees. In Proceedings of the XXVIth International Apicultural Congress, Adelaide. Buchyarest, Romania: Apimodia Publishing House. Pp. 451- 458.

HBRC (Holetta Bee Research Center) 2004.Annual research report.

Heath L. 1985. Occurrence and distribution of chalkbrood disease of honeybees, Bee World, 66: 9-15.

Hijmans, R.J., Cameron S.E., Parra J.L., Jones P.G. and Jarvis A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology. 25: 1965 - 1987.

Hornitzky M. A. (2001) Literature review of chalkbrood- a fungus disease of honey bees (RIRDC report)

MOARD (Ministry of Agriculture and Rural Development) 2005. Annual report.

Nelson, D.L., Barker R.G., Bland S.E., Soehngen U. and Corner J., 1977. Western Canadian chalk brood disease survey of honeybees. American Bee Journal. 117 (8), 494–496,505.

Yakobson B. A., Elad D., Ritter W., Rath W. and Rimeicans J. (2003) Pathological and epidemiological study of chalkbrood disease in Israel, Germany and Thailand. Morphological days in Ceske Budejovica, Proceedings of the International Scientific Conference, Ceske Budejovica, 2003. 21-26.

Zaghloul O.A., Mourad A.K., EL,Kady M.B., Nemat F.M. and Morsy M.E. (2005) Assessment of losses honey in yield due to the chalkbrood disease, with reference to determination of its economic injury levels in Egypt.Commun Agric Appl.Biol.Sci. 70 (4): 703-714.