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Epidemiology, factors influencing prevalence and level of varroosis infestation (*Varroa destructor*) in honeybee (*Apis mellifera*) colonies in different agroecologies of Southwest Ethiopia

Dereje Tulu Robi [a,*], Shiferaw Temteme [a], Melkam Aleme [a], Ararsa Bogale [b], Awraris Getachew [c], Esayas Mendesil [d]

[a] *Ethiopian Institute of Agricultural Research, Tepi Agricultural Research Center, P.O. Box 34, Tepi, Ethiopia*
[b] *Ethiopian Institute of Agricultural Research, Holeta Agricultural Research Center, P.O. Box 2003, Holeta, Ethiopia*
[c] *Department of Animal Sciences, College of Agriculture and Environmental Sciences, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia*
[d] *Department of Horticulture and Plant Sciences, Jimma University College of Agriculture & Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia*

A R T I C L E I N F O

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A B S T R A C T

Little information is available on the epidemiology of varroosis caused by Varroa mite, *Varroa destructor* infestation in Ethiopia, although it is a devastating honeybee disease that results in significant economic losses in beekeeping. Therefore, between October 2021 and October 2022, a cross-sectional study was carried out in different agroecology zones in Southwest Ethiopia to determine the prevalence and associated risk factors for varroosis, as well as the effects of this disease on honeybee colonies and honey production. A multivariate logistic regression analysis was performed to identify possible risk factors for the prevalence of *V. destructor*. A total of 384 adult honeybee and worker or drone brood samples were collected from honeybee colonies and examined using standard diagnostic techniques in the laboratory. The result shows that the prevalence of *V. destructor* was found to be 39.3% (95% CI 34.44–44.21) and 43.2% (38.27–48.18) in adult honeybees and brood, respectively. The major risk factors for the prevalence of *V. destructor* in the study areas included agroecology (OR = 5.2, 95% CI 1.75–14.85), type of hive (OR = 2.9, 95% CI 1.17–17.03), management system (OR = 4.3, 95% CI 1.23–14.70), and colony management (OR = 3.5, 95% CI 1.31–9.14). The lower level of colony infestation in adult bees and brood was measured as 1.97 ± 0.14 and 3.19 ± 0.25, respectively. Season, colony status, colony management, and agroecology were among the determinant factors of the level of varroa mite infestation in adult bees and brood. The results of the study demonstrated that honey production losses are largely attributable to *V. destructor* infestation. Therefore, it is critical to inform the community about the effects of *V. destructor* on honey production and develop and implement effective management strategies for this disease. In addition, further research should be done to identify and isolate additional factors that contribute to varroosis in honeybees in different regions.

* Corresponding author.
E-mail address: derejetulu5@gmail.com (D.T. Robi).

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1. Introduction

Since honeybees (*Apis mellifera*) pollinate both crops and wild plant species, honeybee health is an important concern in beekeeping (Ghazoul, 2005; Muli et al., 2014). Honeybees assist in the conservation of biodiversity and the supply of food through this. As honeybees provide beekeepers with a primary source of income through the sale of hive products, beekeeping can help mitigate poverty in developing countries (Abro et al., 2022; Gratzer et al., 2021). However, a significant challenge for the beekeeping business is posed by varroosis, which are ectoparasites of honeybees. According to Rosenkranz et al. (2010), varroa mites were first found in Asia, a native host. However, the mite made a host shift to the Western honeybee *Apis mellifera* at the beginning of the 20th century and spread to Europe, USA, New Zealand, Africa, and the Middle East from southern and Southwestern Asia during the last century (Roberts et al., 2015; Fazier et al., 2010; Dietemann et al., 2013; Strauss et al., 2013; Muli et al., 2014).

The disease caused by varroa mites in honeybees is called 'varroosis' (Ramsey et al., 2019). The *Varroa destructor*, *V. jacobsoni*, *V. rindereri*, and *V. under woodi* are the four species that have been recognized as existing globally (Dietemann et al., 2013; Mondet et al., 2014; Conte et al., 2020). Infected honeybees suffer major health consequences from *V. destructor*, which is the most prevalent species worldwide (Locke, 2016; Hristov et al., 2020; Galindo-Cardona et al., 2020). The varroa mite feeds on the hemolymph and fat tissues of honeybees throughout their larval and adult stages and causes varroosis. Heavy infection in a colony causes body weight to decrease adult bees' and shorten their lifetime (Rosenkranz et al., 2010). Acute bee paralysis virus (ABPV), Sacbrood virus (SBV), black queen cell virus (BQCV), and deformed wing virus (DWV) are among the honeybee viruses that mites can transmit (Mendoza et al., 2020; Locke et al., 2021).

Furthermore, reports suggest that it serves as a host for bacterial and fungal infections (Hubert et al., 2017). Therefore, the parasitic effects of the varroa mite combined with its impacts on honeybees as a host of various pathogens became one of the main reasons for significant colony losses globally (Martin and Brettell, 2019; Hristov et al., 2020; Bahreini et al., 2020). Furthermore, the quality of bee products is deteriorating as a result of applied acaricides (chemicals used to control mites), which is a great concern, particularly in countries with higher infestation rates (Abd El-Wahab et al., 2021; Bahreini et al., 2020; Qadir et al., 2021). Today, the parasite is found throughout the world, except Australia and New Zealand South Island (FAO, 2009). This mite infests all stages of honeybee development, including larvae, pupae, and adults. Low levels of varroa infestation do not have an obvious effect. Eventually, the mite population reaches a level that the colony cannot tolerate, and thus loses its social organization and disbands, leading to what is known

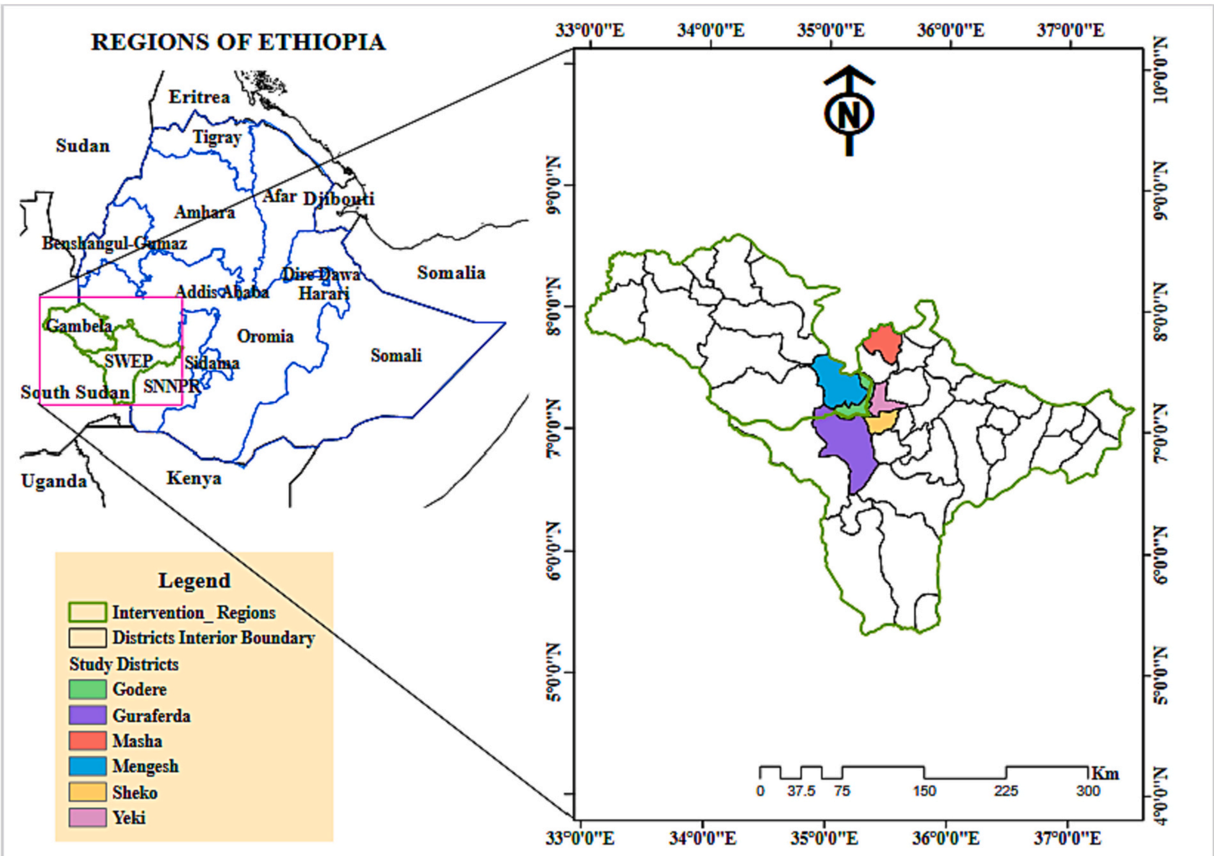

Fig. 1. Map showing the study districts in Southwest Ethiopia.

as colony collapse (Coffey et al., 2010).

Beekeeping is a vital part of the economy of several large settlements in Ethiopia, particularly in the Southwest parts, due to the abundance of suitable natural resources there (Shenkute et al., 2012; Tulu et al., 2020; Tulu et al., 2023). However, the benefits that are currently being reaped are incredibly modest in light of the subsector's potential. Although several factors influence it, the main one is defined as the existence of pests and honeybee diseases. These disease assaults continue to have a serious effect on honeybees and their products, despite the lack of many solid kinds of research on the effects of the disease (Shegaw et al., 2022). Varroosis is one of the serious honeybee diseases that affected honey production in Ethiopia (Begna, 2006; Shegaw et al., 2022; Gela et al., 2023). However, evidence on the magnitude and distribution of varroa mites is still insufficient (Godifey, 2015).

Varroosis leads to significant economic losses, representing a major impediment to honey production (Shimelis, 2017). Most beekeepers and other stakeholders in the areas do not recognize about the varroa mite, its existence, and potential preventive strategies due to its identification challenges combined with a lack of in-depth studies on it. Despite the lack of knowledge, the location and intensity of each economically significant varroosis that affects honeybees have not been thoroughly documented in these areas. Therefore, the objective of this study was to determine the magnitude and associated risk factors for varroosis in different agroecology in Southwest Ethiopia, as well as the effects of this disease on honeybee colonies (*Apis mellifera)* and honey production.

2. Material and methods

2.1. Study areas

The study was carried out in six selected districts in the Sheka, Bench-Sheko, and Majang zones of Southwest Ethiopia, namely the Masha, Yeki, Guraferda, Sheko, Godare and Megesh districts (Fig. 1). These districts were categorized as highland (Masha), midland (Sheko), and lowland (Yeki, Guraferda, Godare, and Mengesh) agroecology zones. The Bench-Sheko and Sheka zones are located Southwest of Addis Ababa, the capital of Ethiopia, at 561 km and 694 km, respectively. The Bench-Sheko zone is located at altitudes ranging from 850 to 3000 m above sea level. The annual average temperature in the Bench-Sheko zone ranged from 20 °C to 40 °C and the annual rainfall ranges from 1200 to 2000 mm. The Sheka zone is located at an altitude ranging from 1200 to 3000 m above sea level. The annual average temperature in the Sheka zone ranges from 15.1 °C to 27.5 °C and the annual mean rainfall ranges from 1201 to 1800 mm. Majang is one of the regional administrative zones of the Gambella Region, and borders the Southwest Ethiopia Region. The zone is characterized by the production of forest coffee along with the spices collected from the forests for the market. Farmers in the Bench-Sheko, Sheka, and Majang zones earn their livelihoods in mixed crop-livestock production systems and beekeeping.

2.2. Study design and period

A cross-sectional study was conducted in the Bench-Sheko, Sheka, and Majang zones from October 2021 to October 2022. The study populations were honeybee colonies in selected districts of study zones with different ages of comb, colony status, agroecology, and management system.

2.3. Sample size and sampling procedure

A multistage purposive random sampling procedure was used to select study zones known for the potentiality of beekeeping. A total of six districts were randomly selected from the three zones, namely Guraferda and Sheko from the Bench-Sheko, Masha and Yeki from the Sheka zone, and Mengesh and Godere from the Majang zones, respectively. A total of 24 kebeles (the smallest administrative units within a district) were randomly selected from the districts. Using a simple random sampling method based on the number of villages in the kebeles, 86 villages were selected from these kebeles. Based on the number of apiaries present in the villages, a simple random sampling method was used to select a total of 138 apiaries. Each honeybee colony in each apiary was taken as a sample using a simple random sampling method. There has been no previous research on varroosis in the study areas. Therefore, the sample size required for the study was determined based on sample size determination in random sampling methods using 50% expected prevalence with a 95% confidence interval at 5% absolute precision following the formula of Thrusfield (2005). Substituted each give 384 honeybee colonies were required for this study.

The state of honeybee colonies can be categorized as treated or untreated. Treated honeybee colonies are those in which certain products and measures are used to maintain the well-being and health of the colonies. In contrast, untreated colonies are those in which minimal or no products are used for colony maintenance. In the study areas, all beekeepers did not use any chemicals or medications to treat their honeybee colonies for managing Varroa mites. This approach is often referred to as organic beekeeping. Nutritional supplements, such as pollen substitutes or protein supplements, were the only products provided to ensure that honeybees have access to a balanced diet (Ahmad et al., 2021; Ghramh and Khan, 2023) in the study areas.

2.4. Laboratory tests of varroosis

About 300–400 adult bees from the brood combs, as well as 300–400 sealed worker or drone brood cell samples, were collected from each colony's brood comb. The honeybee colonies collected from different districts exhibited the following variations: 88 were collected from Guraferda, 92 from Sheko, 69 from Yeki, 42 from Godere, 43 from Mengesh, and 50 from Masha. To preserve them for investigation, the adult bees and the brood that were collected were coded and placed in airtight containers. Adult bees were thoroughly mixed in a flask tube containing a 70% ethyl alcohol solution mixed with 10 ml of 1% detergent water solution for approximately 5 min to remove the phoretic mite. The bees were then filtered through a mesh size of 3–4 mm, which is large enough to let the mites pass through but tiny enough to keep the bees, and then washed. The mite-containing solution was then run through a fine gauze (0.5 mm) with pores small enough to hold it back, after which the mites were carefully counted on the sieves or by turning them down onto white paper (Dietemann et al., 2013). We examined mite populations in the bodies of the larvae, as well as in the sampling broods by carefully removing the larvae from the cell with a pin and opening the cell caps. Infestation level, therefore, is related to the degree to which a colony is impacted by the pest, and can be determined by comparing the count of mites on adult bees or broods that was seen with that count multiplied by 100.

$$\text{Infestation level } (\%) = \frac{\text{Number of varroa mites counted}}{\text{number of tested adult bees or broods}} \times 100$$

2.5. Data collection

Agroecology (highland, midland, and lowland), age of the comb (new or old), type of hive (traditional or framed hive), management system (apiary or backyard), source of the colony (swarming, split or purchasing), season (wet season: June to September or dry season: October to May), colony status (strong, medium, and weak), shade (shaded or open), feeding of the colony during a time of drought (yes or no), honey yield per colony (kg), agrochemicals around the apiary (yes or no) and colony management (managed and unmanaged) were all recorded for each colony. The bee inspector provided an approximate estimate of each colony's strength using three categories: medium colony, weak colony, and strong colony (Morawetz et al., 2019).

2.6. Data management and analysis

The data obtained from the study were recorded and stored in Microsoft® Excel for Windows 2010. Data were then transferred to STATA version 14.0 for Windows (Stata Corp. College Station, TX, USA). The prevalence of varroosis was calculated by dividing the number of colonies positive for the disease by the total number of colonies sampled. The exact Epitools binomial method was used to obtain the 95% confidence interval (CI) for each prevalence of varroosis. ANOVA and *t*-tests were used to compare the mean honey yield and the level of infestation between the colonies positive and negative for varroosis. Honey yield and level of infestation between the varroosis-positive and varroosis-negative colonies in this study were measured as mean values ± standard error (Mean ± SE).

The logistic regression model was used to analyze the association between the prevalence and associated risk factors of *V. destructor* infestation. A screening of several risk factors associated with *V. destructor* infestation was performed using univariate logistic regression analysis. Variables that had a $P \leq 0.25$ in the univariate analysis were further examined in a multivariate analysis. In this analysis, the apiary was added as random effects in the colony models. The variable selection was done based on the backward elimination procedure using an LR-test at 0.05 as the cut point. To test for multicollinearity in the final multivariable models, generalized variable inflation factors (GVIF) were used. Variables having a GVIFˆ (1/2*Df) of >2 were eliminated. A two-way interaction test of biological significance was performed with respect to the variables in the final model.

The effects of possible risk variables for *V. destructor* infection were modeled using multivariate random effects logistic regression. Components in the overall model that were not significant with a *p*-value of less than or equal to 0.05 were removed using the backward elimination procedure. The Hosmer-Lemeshow test was used to observe the model fit. The receiver operating characteristic (ROC) curve was subsequently used to validate the predictive power of the model.

During the analysis, a covariate was considered a confounder and added to the model if it altered the estimated risk OR by >25% (Dohoo et al., 2009). For all statistical analyses, 95% confidence intervals and a critical value of 0.05 were used.

Table 1
Distribution of *Varroa destructor* prevalence in adult bees and brood in Southwest Ethiopia.

		Adult bee	Brood
Study areas	Number of honeybee colonies examined	Prevalence (%) (95%CI)	Prevalence (%) (95%CI)
Guraferda	88	45.5(35.05–55.86)	30.7 (21.05–40.32)
Sheko	92	32.6(23.03–42.19)	42.4 (32.29–52.49)
Yeki	69	26.1(15.73–36.45)	40.6 (28.99–52.17)
Godere	42	47.6(32.51–62.72)	59.5 (44.68–74.37)
Mengesh	43	30.2(16.51–43.96)	53.5 (38.58–68.40)
Masha	50	60.0(46.42–73.58)	48.0 (34.15–61.85)
Overall	384	39.3(34.44–44.21)	43.2 (38.27–48.18)
Chi square value (χ^2)		19.851	19.420
P-value		0.001	0.002

CI: Confidence Interval.

3. Results

3.1. Prevalence of the Varroa destructor in study areas

From 384 honeybee colony samples obtained in Southwest Ethiopia, the overall prevalence of *V. destructor* infestation was 39.3% (95%CI: 34.44–44.21). The prevalence of *V. destructor* infestation was highest in the Masha district (60.0%) and lowest in the Yeki district (26.1%), respectively. The prevalence in both adult bees and the brood showed statistically significant differences ($P < 0.05$) among districts. Furthermore, compared to adult honeybees, the brood was more likely to be infected with *V. destructor* (43.2%) in the study areas (Table 1).

This study shows honey yield in the wet season (17.28 ± 0.63 kg/hive) was significantly lower (P < 0.05) compared to the dry season (18.79 ± 0.70 kg/hive). Moreover, the highest honey yield was recorded in midland area (21.00 ± 0.80 kg/hive), which was less affected by Varroa mites, followed by the highland (20.72 ± 1.54 kg/hive) and lowland (15.93 ± 0.62 kg/hive) areas (Table 2).

3.2. Potential risk factors for varroosis in study areas

In this study, agroecology, comb age, type of hive, colony management, and honey flow season were variables with $P \leq 0.25$ included in multivariate logistic regression analysis. No multicollinearity or significant interactions were found between the variables. The model fits the data, according to a Hosmer-Lemeshow goodness-of-fit value ($\chi^2 = 7.620$, $P = 0.365$). The ROC curve (AUC = 0.757, 95%CI: 0.79–0.92) indicated that the model was accurate. The final multivariate logistic regression model showed that agroecology, type of hive, season, and colony management had a significant ($P < 0.05$) effect on the occurrence of varroosis in honeybee colonies in univariate and multivariate analyses. However, there was no association ($P > 0.05$) between any of the following variables and the prevalence of *V. destructor* infestation: comb age, management system, colony feeding during the dearth period, use of agrochemicals around the apiary site, shading, status or source of a colony (Table 3).

3.3. Infestation level and potential risk factors for varroosis in adult bees and brood stage

The current study showed that the number of mite infestations for both broods and adults was significantly (P < 0.05) associated with agroecology, season, colony status, and colony management (Tables 4 & 5). The levels and factors of infestation, including the age of the comb, the management method, the type of hive, the presence of shading, the feeding colony during the dearth period and the agrochemicals used nearby the apiary did not differ significantly (P > 0.05) in ANOVA and the *t*-test (Tables 4 & 5).

4. Discussion

This study provided epidemiological data on the infestation of *V. destructor* in honeybee colonies in Southwest Ethiopia based on parasitological evidence. The varroa mite in honey bees causes significant economic loss in the country (Gebremedhn et al., 2019; Shegaw et al., 2022). This study helps to apply appropriate management techniques to control and prevent *V. destructor* infestation. Therefore, this study provided information on the epidemiology of *V. destructor* infestation in the areas. This study is essential to provide information to help mitigate and prevent significant diseases (Lee et al., 2015; Silva de Oliveira et al., 2021). The current findings showed that the prevalence of *V. destructor* was 39.3% in adult bees and 43.2% in the brood, respectively. Our results show that honeybee colonies in the study areas have a high level of prevalence of *V. destructor*, resulting in serious economic losses in Southwest Ethiopia. Furthermore, factors such as the type of hive, agroecology, seasons, and colony management affected the occurrence of this disease.

The prevalence of varroosis was highest (60.0%) and lowest (26.1%) in the Masha and Yeki districts, respectively. The difference in prevalence between the studied districts may be due to several factors, including ecological variation, season, and management systems. The overall prevalence of varroosis (39.3%) identified in the current study is similar to the findings of Shegaw et al. (2022), which showed a prevalence of 48.4% in Southwestern Ethiopia. Compared to previous findings in the Tigray region (Begna, 2015), which observed a prevalence of 82% varroosis, the current prevalence is lower. Similarly, a higher prevalence than the current result has also been recorded in other African countries for the same species of honeybee (*Apis mellifera*), including 100% in South Africa (Strauss et al., 2013), 85% in Kenya (Muli et al., 2014), 92% in Tanzania (Mumbi et al., 2014), and 78.6% in Nigeria (Akinwande et al., 2013).The difference in prevalence between locations was caused by interactions among several elements, including ecological conditions, types of honeybees, and varroosis processes (Alattal et al., 2006).

Table 2
Effect of *Varroa destructor* on honey production in different seasons and agroecology of Southwest Ethiopia.

Variables	Category	Negative	Positive	Mean ± SE	Statistics		
Agroecology	Lowland	151	91	15.93 ± 0.62			
	Mid-land	62	30	21.00 ± 0.80	F = 12.427	Df = 2	P = 0.0001
	Highland	20	30	20.72 ± 1.54			
Season	Wet	89	151	17.28 ± 0.63	$t = -755$	Df = 382	P = 0.0004
	Dry	82	62	18.79 ± 0.70			

Table 3
Analysis of potential risk factors for *Varroa destructor* prevalence at the colony level using univariable and multivariable methods in Southwest Ethiopia.

Variables	Category	Total colony examined	Total colony positive (%)	Univariable		Multivariable	
				Crude OR (CI 95%)	P-value	Adjusted OR (CI 95%)	P-value
Agroecology					0.005		0.002
	Lowland	242	37.6	2.5(1.34–4.64)	0.004	3.2(1.63–6.16)	0.001
	Mid-land	92	32.6	3.1(1.52–6.33)	0.002	3.1(1.39–6.83)	0.006
	Highland	50	60.0	–	–	–	–
Colony source					0.311		
	Swarming	344	40.4	0.8(0.34–2.06)	0.708		
	Purchasing	18	22.2	2(0.49–8.20)	0.335		
	Splitting	22	36.4	–	–		
Age of comb	Old	274	36.5	1.5(0.96–2.36)	0.074		
	New	110	46.4	–	–		
Management system	Apiary	308	39.3	1.0(0.60–1.69)	0.976		
	Backyard	76	39.5	–	–		
Type of hive	Framed hive	256	36.3	2.5(1.94–3.25)	0.090	2.6(1.99–3.64)	0.035
	Traditional	128	45.3	–	–	–	–
Colony management	Unmanaged	234	34.2	2.7(2.14–3.63)	0.010	2.6(1.99–3.84)	0.026
	Manage	150	47.3	–	–	–	–
Shading	Shaded	221	38.9	1.0(0.69–1.58)	0.849		
	Open	163	39.9	–	–		
Colony status					0.341		
	Medium	68	41.2	1(0.56–1.65)	0.884		
	Weak	30	26.7	1.8(0.80–4.30)	0.153		
	Strong	286	40.2	–	–		
Feeding colony during dearth	Yes	78	53.9	1.2(0.54–2.55)	0.691		
	No	306	35.6	–	–		
Agrochemical around apiary	Yes	332	39.5	1.1(0.57–1.90)	0.891		
	No	52	38.5	–	–		
Season	Wet	216	43.5	1.5(1.0–2.28)	0.057	1.6(1.02–2.44)	0.039
	Dry	168	33.9	–	–	–	–

OR: Odds Ratio; CI: Confidence Interval.

Table 4
Analysis of potential risk factors for *Varroa destructor* infestation level in adult bees.

Factors	Category	N	Mean ± SE	Statistics		
Agroecology	Lowland	242	1.23 ± 0.17			
	Mid-land	92	0.68 ± 0.13	F = 9.402	Df = 2	P = **0.0001**
	Highland	50	2.74 ± 0.62			
Colony source	Swarming	344	1.37 ± 2.91			
	Purchasing	18	1.13 ± 0.27	F = 1.42	Df = 2	*P* = 0.244
	Splitting	22	0.69 ± 0.25			
Age of comb	Old	274	1.19 ± 0.16	*t* = 1.162	Df = 382	*P* = 0.246
	New	110	1.55 ± 0.29			
Management system	Apiary	224	1.17 ± 0.17	*t* = 0.991	Df = 382	*P* = 0.322
	Backyard	160	1.46 ± 0.25			
Type of hive	Framed hive	256	1.19 ± 0.16	*t* = 1.028	Df = 382	*P* = 0.304
	Traditional	128	1.50 ± 0.29			
Colony management	Unmanaged	234	1.99 ± 0.31	*t* = 4.052	Df = 382	P = **0.0001**
	Manage	150	0.84 ± 0.11			
Shading	Shaded	221	1.10 ± 0.16	*t* = 1.609	Df = 382	*P* = 0.108
	Open	163	1.56 ± 0.26			
Colony status	Medium	140	0.83 ± 0.56			
	Weak	30	0.63 ± 0.21	F = 5.058	Df = 2	*P* = **0.007**
	Strong	214	1.70 ± 0.23			
Feeding colony during dearth	Yes	78	1.06 ± 0.31	*t* = 460	Df = 382	*P* = 0.646
	No	306	1.31 ± 0.18			
Agrochemicals around apiary	Yes	332	1.37 ± 0.16	*t* = 1.334	Df = 382	*P* = 0.183
	No	52	0.81 ± 0.18			
Season	Wet	216	1.74 ± 0.29	*t* = 2.462	Df = 382	*P* = **0.014**
	Dry	168	1.02 ± 0.14			

N = number of colonies.

Table 5
Analysis of potential risk factors for *Varroa destructor* infestation level in brood cells.

Factors	Category	N	Mean ± SE	Statistics		
Agroecology	Lowland	242	3.64 ± 0.34			
	Mid-land	92	1.76 ± 0.37	F = 5.145	Df = 2	*P* = **0.006**
	Highland	50	3.60 ± 0.69			
Colony source	Swarming	344	3.44 ± 0.28			
	Purchasing	18	1.19 ± 0.35	F = 4.492	Df = 2	*P* = **0.012**
	Splitting	22	1.19 ± 0.35			
Age of comb	Old	274	2.89 ± 0.45	*t* = −749	Df = 382	*P* = 0.454
	New	110	3.31 ± 0.31			
Management system	Apiary	224	2.82 ± 0.32	*t* = 1.704	Df = 382	*P* = 0.089
	Backyard	160	3.69 ± 0.41			
Type of hive	Framed hive	256	1.19 ± 0.16	t = 1.028	Df = 382	P = 0.304
	Traditional	128	1.50 ± 0.29			
Colony management	Unmanaged	234	4.45 ± 0.47	t = 4.052	Df = 382	P = **0.0001**
	Manage	150	2.37 ± 0.27			
Shading	Shaded	221	2.76 ± 0.32	*t* = 1.955	Df = 382	*P* = 0.057
	Open	163	3.76 ± 0.41			
Colony status	Medium	140	2.52 ± 0.35			
	Weak	30	1.99 ± 0.71	F = 3.796	Df = 2	*P* = **0.023**
	Strong	214	3.79 ± 0.37			
Feeding colony during dearth	Yes	78	3.20 ± 0.94	*t* = 0.018	Df = 382	*P* = 0.986
	No	306	3.18 ± 0.26			
Agrochemicals around apiary	Yes	332	3.50 ± 0.29	*t* = 1.147	Df = 382	*P* = 0.252
	No	52	1.20 ± 0.22			
Season	Wet	216	3.56 ± 0.45	*t* = 3.155	Df = 382	*P* = **0.002**
	Dry	168	2.96 ± 0.30			

N = number of colonies.

Several studies conducted in Ethiopia have examined the prevalence of Varroa infestation in honeybee colonies and reported a high prevalence ranging from 30.5% to 91.8%. Furthermore, the infestation has been reported to have significant detrimental effects on both honeybee colonies and honey production, posing a considerable challenge for the country's beekeeping industry (Begna, 2015; Godifey, 2015; Mezgabu et al., 2016; Nega et al., 2019; Shegaw et al., 2022; Gela et al., 2023). Furthermore, studies have shown that Ethiopian honey bee antennae exhibit an increased level of gene expression of the odorant binding protein OBP14, suggesting a potential improvement in detection and elimination of reproducing mites (Gebremedhn et al., 2023).

In this study, a higher prevalence (43.2%) of *V. destructor* was observed in the brood than in adult honeybees (39.3%). Although *V. destructor* infested adult honeybee and broods, the presence of broods is essential for the survival of this parasite. Since the adult female mite produces eggs and phoretic mites fully mature in broods (Conte et al., 2020; Underwood and Lopez-Uribe, 2022). Consequently, the strength of a colony (the presence of a prodigious queen and a large number of foragers and drones) is closely associated with the amount of brood in that colony, and the availability of abundant forages sources plays a significant role in determining this association (El-Niweiri and El- Sarrag, 2006). This result is in line with previous research conducted in Ethiopia (Shegaw et al., 2022), which found a higher prevalence of varroosis in brood than in adult honeybees. Similarly, to this, the brood had much higher mean levels of infection than adult bees. This supports the findings of Shegaw et al. (2022) and El-Niweiri and El- Sarrag (2006), who found that the levels of *V. destructor* infestation varied between the brood and adult bees in Ethiopia and Sudan, respectively.

The high infestation rate in both the brood and adult bees recorded in this study could attributed to several factors. Environmental stress may play a significant role in increasing the susceptibility of honeybee colonies to mite infestations (El-Seedi et al., 2022). Previous research reports revealed that pesticide exposure, habitat loss, climate change, and nutritional deficiencies could contribute to the weakening of honeybee colonies (Manzoor and Pervez, 2022). Pesticides, in particular, disrupt the immune systems of honeybees, making them more susceptible to mite infestations (Harwood and Dolezal, 2020). In addition, habitat loss and changes in the availability of nectar and pollen sources can result in weakened bees with compromised resistance to infestations (Belsky and Joshi, 2019; de Jongh et al., 2022). Inadequate beekeeping practices, infestations, including poor hive management, insufficient pest monitoring, and inadequate sanitation, further exacerbate the problem of mite infestations. Poor hive management, insufficient pest monitoring, and inadequate sanitation by creating favorable conditions for mites to thrive (Wakgari and Yigezu, 2021). Neglecting regular hive inspections of the hive and failing to take timely action against infestations allow mite populations to grow unchecked, leading to severe infestations that are difficult to control.

Varroosis reduced the honey yield per colony, with a statistically significant difference between seasons ($P = 0.0004$) and agroecology ($P = 0.0001$) and a loss of honeybee production. This might be the case since agroecology directly affects ecological variables, including vegetation, temperature, and humidity, all of which have an impact on nectar flow, the time it takes for a brood to grow, and the survival and reproduction rates of mites. It is probable that the poor brood-rearing techniques used during the dry season, which limit the parasites' ability to develop and multiply, are responsible for the increased honey yield loss caused by *V. destructor* infestation in the wet season. Varroosis is currently the most damaging honeybee parasite (Rosenkranz et al., 2010), supporting this finding. The obligatory parasite *V. destructor* is also the most destructive pest to honeybee colonies globally, as it can infect *A. mellifera* at different stages of growth and castes (Shen et al., 2005).

Lowland colonies are 5.2 times more likely to be infested with varroa mites than highland colonies, and there is a significant association between the prevalence of varroosis and agroecology ($P < 0.05$). This may be due to the fact that agroecology directly influencing ecological variables including vegetation, temperature, and humidity, all of which have an impact on nectar flow, the period during which the brood develops, and the survival and reproduction rates of mites (Pedro et al., 2015). This result supports a previous finding (Shegaw et al., 2022) that agroecology poses a major risk to the development of varroosis in honeybee colonies in Ethiopia. This also supports the research by Muli et al. (2014), who found that seasonal variation, elevation, and geographic area had a significant impact on the level of the varroa mite, indicating that environmental factors could control the rate of infection with varroosis.

The type of hive was found to be significantly ($P < 0.05$) associated with the prevalence of varroosis, with framed hives (modern hive types) having an odds ratio of 2.9, which is almost three times higher than traditional hive types (skep, log hive, or clay pot hive). During harvest, all resources (honey, brood, and pollen) are completely removed from traditional hives to trap swarms. This is often done a few days or months before the start of the main blooming seasons. This most likely makes it difficult for the phoretic mite to access broods for extended periods and reproduce. Furthermore, because colonies are spread out, they are less likely to be contaminated by robbery and drifting. In light of this, these factors probably explain the low incidence of mites in forests as opposed to in backyards and apiary locations. This supports previous findings in Ethiopia by Gebremedhn et al. (2019) and Shegaw et al. (2022) who reported that the infestation of *V. destructor* varied depending on the type of hive used. Similarly, colony management was also significantly associated ($P < 0.05$) with the occurrence of varroosis in the areas studied. The likelihood of the parasite *V. destructor* infestation was almost four times (OR = 3.5) higher in honeybee colonies kept in unmanaged areas compared to colonies managed in clear areas. This is in agreement with other studies that showed that honeybees kept in unmanaged areas had higher infestation rates of *V. destructor* (Shegaw et al., 2022).

In this study, a significant association was also found between the season and the occurrence of varroosis in honeybee colonies. The probability of varroosis was about two (OR = 1.6) times higher in the wet season compared to the dry season. The higher prevalence of *V. destructor* infestation in the wet season may be due to poor brood-rearing practices during the dry season, which limit the ability of the parasites to grow and reproduce. This could be justified by high temperatures in dry season may limit the development of mites in the brood, and there is also a stoppage of egg-laying by the queen, resulting in a decrease in the growth of varroa in bee colonies (Kablau et al., 2020). The risk factors that significantly affected patterns in the varroa mite population included the length of absence of brood, the number of phoretic mites, honeybee reproduction, mite reproduction, and mite mortality (Piou et al., 2016). The findings of this study support previous research in Ethiopia that *V. destructor* infestation is significantly associated with season (Gemedi, 2017; Shegaw et al., 2022). Consistent with this finding of Rosenkranz et al. (2010), who showed that the broodless phase reduced the growth of the mite population. Another study has revealed that Varroa populations reach their peak during the warm and wet seasons, suggesting a correlation between environmental conditions and mite reproduction (Begna et al., 2016; Gebremedhn et al., 2019).

The levels of Varroa mite infestation in the study areas were 1.972% in adult bees and 3.186% in brood, respectively. This finding was similar to previously reported levels of 1.924% in south-western Ethiopia (Shegaw et al., 2022) and 2.67% in the Toke-Kutaye district, West Shoa Zone (Mengistu et al., 2016). Fazier et al. (2010) also noted 3.67% in Kenya, which is in line with the present findings. However, the observed results were lower than the infection levels of 15.73% in adult bees and 18.07% in the brood recorded in central Ethiopia (Mezgabu et al., 2016). Environmental factors and colony management techniques could be responsible for the increase in infestation in different locations. The *Apis mellifera bandansi* and *A.m. monticola* races of honeybees are different from the *Apis mellifera scutellata* (Southwest Ethiopia) races in that they are found in the central, and northern parts of the country, respectively, and are characterized by less hygienic behaviors. This result is lower than the temperate bee, due to their unique grooming and hygiene behaviors, tropical (Africanized) honey bees are more resistant to varroa mites than temperate bees (Mendoza et al., 2020; Pirk et al., 2016). Similarly, tropical honey bees exhibit lower levels of varroa mite infestation compared to temperate honey bees for many reasons, including shorter durations of brood emergencies, high suppression efficiency on mite reproductive successes, and sealing of contaminated drone broods (El-Niweiri and El- Sarrag, 2006; Mendoza et al., 2020; Conte et al., 2020).

According to research in southwestern Ethiopia (Shegaw et al., 2022) and the Tigray region (Haftom et al., 2019), infection levels were higher during the wet season than during the dry season. Lower temperatures and higher humidity during the wet season that influenced the hygienic behavior of honey bees that is inversely correlated with the level of infestation may be the cause of the higher level of infection during this season (Masaquiza et al., 2021). Similarly, the level of infection was also strongly correlated with the strength of the colony and the agroecology of the area where the colony was located, both in adult bees and in brood cells. This confirms other studies (Muli et al., 2014; Gratzer et al., 2021; Dessalegn et al., 2016; Shegaw et al., 2022) that the level of infestation of varroa mites is strongly correlated with colony strength and agroecology. This could be influenced by various environmental elements, including climate, colony density, feed availability, and management, as well as brood characteristics such as attractiveness, stimulation, post-caping behavior, and grooming behavior (Mancuso et al., 2020; Nganso et al., 2017; Tsuruda et al., 2012).

The level of infestation in adult bees and brood was significantly associated with colony management, with a higher mean infestation level found in unmanaged colonies. This was explained by the fact that colony management techniques, such as removing empty combs and other debris from hives, can create possibilities for mite removal by preventing them from being hidden longer in the combs. A small number of beekeepers also claimed that they eliminated or decreased the number of brood combs and smoked herbs during its extreme incidences. In line with this result, Shegaw et al. (2022) and Hillayova et al. (2022) showed that the use of an effective colony management approach considerably decreased the degree of mite infection, as it affected the number of varroa mites that were dying of the bees.

Curie (2008) and Paray and Gupta (2017) reported the same inconsistent results when assessing the minimum economic threshold infestation levels of varroa mites due to the impact of the season and various infections. However, in light of these results, colonies with an infection level of 2% measured by ethyl alcohol wash tests are more likely to be considered to have reached minimum threshold levels (Jack and Ellis, 2021; Spivak and Reuter, 2016). In light of this, the present study revealed that 38 (26.39%) and 39 (16.25%) colonies as having >2% infection levels during the dry and wet seasons, respectively.

The study of varroa mites faced limitations with respect to facilities and resources and, as a result, molecular methods could not be employed. Given this constraint, future research on the varroa mite should employ molecular methods to further investigate the subject. This will ensure that more comprehensive information is obtained, which can then be utilized to devise more effective measures to manage the population of varroa mites. Using the latest molecular techniques, researchers can conduct a more detailed examination of the mite's biology and genetics, providing an enhanced understanding of its behavior and interactions with its environment. However, the findings of this research can contribute to the development of more accurate and focused control strategies to limit the damage caused by the varroa mite.

5. Conclusions

The findings revealed a high prevalence of *V. destructor* in adult honeybees and brood in different agroecology zones in Southwest Ethiopia. Several risk factors were identified, including agroecology, type of hive, management system, and colony management, which significantly influenced the prevalence of *V. destructor*. In addition, the study measured the level of *V. destructor* infestation in adult bees and brood and found lower levels of infestation. Factors such as season, colony status, colony management, and agroecology were found to be determinants of the level of *V. destructor* infestation. These results underscore the substantial impact of *V. destructor* infestation on honey production. Given the importance of *V. destructor* in honeybee colonies, it is crucial to raise awareness among the community about the detrimental effects of this disease on honey production. Effective management strategies should be developed and implemented to mitigate the impact of *V. destructor*. Furthermore, future research should focus on identifying and isolating additional factors that contribute to varroosis in honeybees in different regions.

Ethical statement

The procedures conducted in this study were strictly in accordance with the experimental protocols and ethical standards established by the Animal Welfare and Research Ethics Committee of the Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa. These protocols were based on the principles of global animal welfare, ensuring that the research was conducted ethically and responsibly. Furthermore, verbal consent was obtained from all beekeepers and they were assured that their participation would be kept confidential. This approach secured the full cooperation and voluntary participation of each beekeeper involved in the study.

Declaration of Competing Interest

The authors have not declared any conflicts of interest.

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References

Abd El-Wahab, T.E., Shalaby, S.E., Al-Kahtani, S.N., Al Naggar, Y., Jamal, Z.A., Masry, S.H., 2021. Mode of application of acaricides against the ectoparasitic mite (*Varroa destructor*) infesting honeybee colonies, determines their efficiencies and residues in honey and beeswax. J. King Saud Univ. Sci. 33 (1), 101236.

Abro, Z., Kassie, M., Tiku, H.A., Taye, B., Ayele, Z.A., Ayalew, W., 2022. The impact of beekeeping on household income: evidence from north-western Ethiopia. Heliyon 8 (5), e09492. https://doi.org/10.1016/j.heliyon. 2022.e09492.

Ahmad, S., Khan, K.A., Khan, S.A., Ghramh, H.A., Gul, A., 2021. Comparative assessment of various supplementary diets on commercial honey bee (*Apis mellifera*) health and colony performance. PLoS One 16 (10), e0258430. https://doi.org/10.1371/journal.pone.0258430.

Akinwande, K.L., Badejo, M.A., Ogbogu, S.S., 2013. Challenges associated with the honey bee (*apis mellifera adansonii*) colonies establishment in south western Nigeria. Afr. J. Food Agric. Nutr. Dev. 13 (2), 1–18.

Alattal, Y., Rosenkranz, P., Claus, P., Zebitz, W., 2006. Infestation levels of *Varroa destructor* in local honey bees of Jordan. Mitt. Dtsch. Ges. Allg. Angew. Ent. 15, 321–326.

Bahreini, R., Nasr, M., Docherty, C., de Herdt, O., Muirhead, S., Feindel, D., 2020. Evaluation of potential miticide toxicity to Varroa destructor and honey bees, *Apis mellifera*, under laboratory conditions. Sci. Rep. 10 (1), 1–14.

Begna, D., 2006. The occurrence of Chalk brood (*Ascosphaera apis*): A new honeybee (*A. mellifera L.*) disease in West Shoa, Ethiopia. Ethiopian J. of Ani. Prod. 6 (1), 1–8.

Begna, D., 2015. Occurrences and distributions of honeybee (*Apis mellifera Jemenetica*) varroa mite (*Varroa destructor*) in Tigray region, Ethiopia. J. Fish. Livestock Product. 3, 126. https://doi.org/10.4172/2332-2608.1000126.

Begna, D., Gela, A., Negera, T., Bezabeh, A., 2016. Identifying the species, effects and seasonal dynamics of honeybee Varroa mites: a newly emerging parasite to Ethiopian honeybee. Int. J. Toxicol. Environ. Sci. 1 (1), 4.

Belsky, J., Joshi, N.K., 2019. Impact of biotic and abiotic stressors on managed and feral bees. Insects 10, 233. https://doi.org/10.3390/insects10080233.

Coffey, M.F., Breen, J., Brown, M.J.F., McMullan, J.B., 2010. Brood-cell size has no influence on the population dynamics of Varroa destructor mites in the native western honey bee, *Apis mellifera mellifera*. Apidologie. 41, 522–530.
Conte, Y.L., Meixner, M.D., Brandt, A., Carreck, N.L., Costa, C., Mondet, F., Buchler, R., 2020. Review on geographical distribution and selection of European honey bees resistant to *Varroa destructor*. J. Insects 11 (12), 873.
Curie, 2008. Economic threshold for Varroa on the Canadian prairies. In: Canadian Association of Professional Apiculturists. University of Manitoba, Department of Entomology, p. 2N2.
Dessalegn, B., Alemayehu, G., Taye, N., Amssalu, B., 2016. Identifying the species, effects and seasonal dynamics of honeybee varroa mites: A newly emerging parasite to Ethiopian honeybee. Int. J. Toxicol. Environ. Sci. 1 (1), 4. https://doi.org/10.15226/2572-3162/2/1/00102.
Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S., Wauquiez, Q., Tannahil, C., Frey, E., Ziegelmann, B., Rosenkranz, P., Ellis, J.L., 2013. Standard methods for varroa research. J. Apic. Res. 52 (1), 1–54.
Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd ed. AVC, Charlottetown, Prince Edward Island, pp. 239–249.
El-Niweiri, M.A.A., El- Sarrag, M.S.A., 2006. Detection of the parasitic mite (*Varroa jacobsoni*) of honey bees (*Apis mellifera*) in Sudan. Albuhuth 10 (1), 61–76.
El-Seedi, H.R., Ahmed, H.R., El-Wahed, A.A.A., Saeed, A., Algethami, A.F., Attia, N.F., Guo, Z., Musharraf, S.G., Khatib, A., Alsharif, S.M., Naggar, Y.A., Khalifa, S.A. M., Wang, K., 2022. Bee stressors from an immunological perspective and strategies to improve bee health. Vet. Sci. 9 (5), 199. https://doi.org/10.3390/vetsci9050199.
FAO, 2009. Bees and their role in forest livelihoods: a guide to the services provided by bees and the sustainable harvesting, processing and marketing of their products by Nicola Bradbear. Paper 19, 204.
Fazier, M., Muli, E., Conklin, T., Schmehl, D., Torto, B., Frazier, J., Tumlinson, J., Evans, J.D., Raina, S., 2010. A scientific note on *Varroa destructor* found in East Africa; threat or opportunity? Apidology 41 (4), 463–465.
Galindo-Cardona, A., Scannapieco, A.C., Russo, R., Escalante, K., Geria, M., Lepori, N., Monmany-Garzia, A.C., Muntaabski, I., Liendo, M.C., Landi, L., Giray, T., Monmany-Garzia, A.C., 2020. *Varroa destructor* parasitism and genetic variability at honey bee (*Apis mellifera*) drone congregation areas and their associations with environmental variables in Argentina. Front. Ecol. Evol. 8, 590345.
Gebremedhn, H., Amssalu, B., Smet, L.D., de Graaf, D.C., 2019. Factors restraining the population growth of *Varroa destructor* in Ethiopian honey bees (*Apis mellifera simensis*). PLoS One 14 (9), e0223236.
Gebremedhn, H., Claeys Bouuaert, D., Asperges, M., Amssalu, B., De Smet, L., de Graaf, D.C., 2023. Expression of molecular markers of resilience against *Varroa destructor* and bee viruses in Ethiopian honey bees (*Apis mellifera simensis*) focusing on olfactory sensing and the RNA interference machinery. Insects 14, 436. https://doi.org/10.3390/insects14050436.
Gela, A., Atickem, A., Bezabeh, A., Woldehawariat, Y., Gebresilassie, A., 2023. Insights into varroa mite (*Varroa destructor*) infestation levels in local honeybee (*Apis mellifera*) colonies of Ethiopia. J. Appl. Entomol. 00, 1–11. https://doi.org/10.1111/jen.13171.
Gemedi, D., 2017. Status of honeybee *Apis mellifera Bandansii* pests and pathogens in seka Chokersa District of Jimma zone, Ethiopia. Int. J. Res. Stud. Biosci. (IJRSB) 5 (12), 12–27.
Ghazoul, J., 2005. Buzziness as usual? Questioning the global pollination crisis. Trends Ecol. Evol. 20 (7), 367–373.
Ghramh, H.A., Khan, K.A., 2023. Honey bees prefer pollen substitutes rich in protein content located at short distance from the apiary. Animals 13, 885. https://doi.org/10.3390/ani1305088.
Godifey, G., 2015. Epidemiology of Honey Bee Disease and Pests in Selected Zones of Tigray Region, Northern Ethiopia. M.Sc. Thesis. Bahir Dar University, pp. 1–68.
Gratzer, K., Wakjira, K., Fiedler, S., Brodschneide, R., 2021. Challenges and perspectives for beekeeping in Ethiopia. A review. Agron. Sustain. Dev. 41, 46. https://doi.org/10.1007/s13593-021-00702-2.
Haftom, G., Amssalu, B., Smet, L.D., De Graaf, D.C., Lihoreau, M., 2019. Factors restraining the population growth of Varroa destructor in Ethiopian honey bees (*Apis mellifera simensis*). PLoS ONE 14 (9), e0223236. https://doi.org/10.1371/journalpone.0223236.
Harwood, G.P., Dolezal, A.G., 2020. Pesticide-virus interactions in honey bees: challenges and opportunities for understanding drivers of bee declines. Viruses 12 (5), 566. https://doi.org/10.3390/v12050566.
Hillayova, M.K., Korený, L., Skvarenina, J., 2022. The local environmental factors impact the infestation of bee colonies by mite Varroa destructor. J. Ecol. Indicat. 141, 109104.
Hristov, P., Shumkova, R., Palova, N., Neov, B., 2020. Factors associated with honey bee colony losses: a mini-review. J. Vet. Sci. 7 (4), 166.
Hubert, J., Bicianova, M., Ledvinka, O., Kamler, M., Lester, P.J., Nesvorna, M., Erban, T., Erban, T., 2017. Changes in the bacteriome of honey bees associated with the parasite *Varroa destructor*, and pathogens Nosema and Lotmaria passim. J. Microb. Ecol. 73 (3), 685–698.
de Jongh, E.J., Harper, S.L., Yamamoto, S.S., Wright, C.J., Wilkinson, C.W., Ghosh, S., Otto, S.J.G., 2022. One health, one hive: a scoping review of honey bees, climate change, pollutants, and antimicrobial resistance. PLoS One 17 (2), e0242393. https://doi.org/10.1371/journal.pone.0242393.
Jack, C.J., Ellis, J.D., 2021. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera L.* (Hymenoptera: Apidae)) Colonies. J. Insect Sci. 21 (5), e0223236 https://doi.org/10.1093/jisesa/ieab058.
Kablau, A., Berg, S., Hartel, S., Scheiner, R., 2020. Hyperthermia treatment can kill immature and adult *Varroa destructor* mites without reducing drone fertility. Apidologie 51, 307–315. https://doi.org/10.1007/s13592-019-00715-7.
Lee, K.V., Steinhauer, N., Rennich, K., Wilson, M.E., Tarpy, D.R., Caron, D.M., Rose, R., Delaplane, K.S., Baylis, K., Lengerich, E.J., Pettis, J., Skinner, J.A., Wilkes, J.T., Sagili, R., vanEngelsdorp, D., 2015. A national survey of managed honey bee 2013-2014 annual colony losses in the USA. Apidologie 46 (3), 292–305.
Locke, B., 2016. Natural Varroa mite-surviving *Apis mellifera* honey bee populations. Apidologie 47 (3), 467–482.
Locke, B., Thaduri, S., Stephan, J.G., Low, M., Blacquière, T., Dahle, B., Le Conte, Y., Neumann, P., de Miranda, J.R., 2021. Adapted tolerance to virus infections in four geographically distinct Varroa destructor resistant honeybee populations. Sci. Rep. 11 (1), 1–12.
Mancuso, T., Croce, L., Vercelli, M., 2020. Total brood removal and other bio techniques for the sustainable control of varroa mites in honey bee colonies: economic impact in beekeeping farm case studies in northwestern Italy. J. Sustain. 12 (6), 2302.
Manzoor, F., Pervez, M., 2022. Pesticide Impact on Honeybees Declines and Emerging Food Security Crisis. Global Decline of Insects. IntechOpen. https://doi.org/10.5772/intechopen.98871.
Martin, S.J., Brettell, L.E., 2019. Deformed wing virus in honey bees and other insects. Annu. Rev. Virol. 6, 49–69.
Masaquiza, D., Vargas, J., Ortíz, N., Salazar, R., Curbelo, L., Pérez, A., Arenal, A., 2021. Hygienic, behavior of *Apis mellifera* and its relationship with *Varroa destructor*. Infestation and honey production in the Central Highlands of Ecuador. Insects 12, 966.
Mendoza, Y., Tomasco, I.H., Antúnez, K., Castelli, L., Branchiccela, B., Santos, E., Invernizzi, C., 2020. Unraveling honeybee *Varroa destructor* interaction: multiple factors involved in differential resistance between two Uruguayan populations. J. Vet. Sci. 7, 116.
Mengistu, S., Kebede, Y., Begna, D., 2016. Major honeybee health problem with particular emphasis to anti-Varroa investigation of Propolis in toke-Kutaye District. Ethiopia. Am. Eurasian J. Sci. Res. 11 (5), 320–331.
Mezgabu, E., Hirpa, E., Begna, D., Yimer, L., Bayan, A., Chali, M., 2016. Occurrence and distribution of Varroa mite and antivarroa effect of propolis in Walmara District of Oromia special zone around Finfine, Ethiopia. J. Vet. Sci. Technol. 7 (5), 370.
Mondet, F., de Miranda, J.R., Kretzschmar, A., Le Conte, Y., Mercer, A.R., Schneider, D.S., 2014. On the front line: quantitative virus dynamics in honeybee (*Apis mellifera L.*) colonies along a new expansion front of the parasite Varroa destructor. PLoS Pathog. 10 (8), e1004323.
Morawetz, L., Köglberger, H., Griesbacher, A., Derakhshifar, I., Crailsheim, K., Brodschneider, R, Moosbeckhofer, R., 2019. Health status of honey bee colonies (*Apis mellifera*) and disease-related risk factors for colony losses in Austria. PLoS ONE 14 (7), e0219293. https://doi.org/10.1371/journal.pone.0219293.
Muli, M., Harland, P., Maryann, F., James, F., Baldwyn, T., Tracey, B., Joseph, K., James, N.K., Fiona, M., Daniel, M., James, T., Christina, G., 2014. Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (*Apis mellifera*) populations in East Africa. PLoS One 9 (4), 1–11.
Mumbi, C.T., Angela, R.M., Issa, H.M., Allen, R., Raphael, M., 2014. Parasitic mite, Varroa species (Parasitiformes: Varroidae) infesting the colonies of African honeybees, *Apis mellifera scutellata* (Hymenoptera: Apididae) in Tanzania. J. Entomol. Zool. Stud. 2 (3), 188–196.

Nega, T., Yayeh, M., Mitiku, T., Birhan, M., Bogale, B., Chanie, M., Kinubeh, A., 2019. Prevalence of parasitic honeybee diseases, pests and predators in North Gondar zone. Online J. Anim. Feed Res. 9 (5), 198–205.

Nganso, B.T., Fombong, A.T., Yusuf, A.A., Pirk, C.W.W., Stuhl, C., Torto, B., Blenau, W., 2017. Hygienic and grooming behaviors in African and European honeybees—new damage categories in *Varroa destructor*. PLoS One 12 (6), e0179329.

Paray, M., Gupta, S., 2017. Economic threshold of *Varroa destructor* (Anderson and Trueman) infesting *Apis mellifera* in Kashmir. Indian J. Entomol. 79 (1), 27–31.

Pedro, S., Priscila, W., André, L., Patrícia, F., Vagner, A.T., 2015. *Varroa destructor* mite in Africanized honeybee colonies *Apis mellifera L.* under royal jelly or honey production. Acta Sci. 37 (3), 315–322.

Piou, V., Tabart, J., Urrutia, V., Hemptinne, J.-L., Vétillard, A., 2016. Impact of the Phoretic phase on reproduction and damage caused by *Varroa destructor* to its host, the European honey bee (*Apis mellifera* L.). PLoS One 11 (4), e0153482.

Pirk, C.W.W., Strauss, U., Yusuf, A.A., Demares, F., Human, H., 2016. A review on: Honeybee health in Africa. Apidologie 47 (3), 276–300. https://doi.org/10.1007/s13592-015-0406-6.

Qadir, Z.A., Idrees, A., Mahmood, R., Sarwar, G., Bakar, M.A., Ahmad, S., Raza, M.M., Li, J., 2021. Effectiveness of different soft acaricides against honey bee ectoparasitic mite *Varroa destructor* (Acari: Varroidae). Insects 12 (11), 1032.

Ramsey, S.D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J.D., Cohen, A., vanEngelsdorp, D., Joklik, J., Cicero, J.M., Ellis, J.D., Hawthorne, D., vanEngelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and nothemolymph. Proc. Natl. Acad. Sci. 116 (5), 1792–1801.

Roberts, J.M.K., Anderson, D.L., Tay, W.T., 2015. Multiple host shifts by the emerging honeybee parasite, *Varroa jacobsoni*. Mol. Ecol. 24, 2379–2391.

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103, 96–119.

Shegaw, T., Arke, A., Belay, N., Giorgis, D.H., 2022. Diagnostic survey on varroa mite (*Varroa distractor*) prevalence in South-Western Ethiopia. Cogent. Food Agricult. 8 (1), 2143610.

Shen, M., Cui, L., Ostiguy, N., Cox-Foster, D., 2005. Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and sacbrood virus) with the honeybee host and the parasitic varroa mite. J. Gen. Virol. 86, 2281–2289.

Shenkute, A.G., Getachew, Y., Assefa, D., Adgaba, N., Ganga, G., Abebe, W., 2012. Honey production systems (*Apis mellifera L.*) in Kaffa, Sheka and bench-Maji zones of Ethiopia. J. Agricult. Extens. Rural Dev. 4 (19), 528–541.

Shimelis, S., 2017. Survey of Honey Production System and Honey Bee Disease and Pests in Ejere District, West Shewa Zone, Oromia National Regional State, Ethiopia. MVSc Thesis. Addis Ababa University, College of Veterinary Medicine and Agriculture, pp. 1–39.

Silva de Oliveira, V.H., Nilsson, A., Kim, H., Hallgren, G., Frossling, J., Kristiansen, L.F., Forsgren, E., 2021. Honey bee pathogens and parasites in Swedish apiaries: a baseline study. J. Apic. Res. 60 (5), 697–706.

Spivak, M., Reuter, G.S., 2016. Honeybee Diseases and Pests: A Companion to Beekeeping in Northern Climate. University of Minnesota Extension, Department of Entomology and Minnesota Extension Service, St. Paul.

Strauss, U., Human, H., Gauthier, L., Crewe, R.M., Dietemann, V., Pirk, C.W., 2013. Seasonal prevalence of pathogens and parasites in the savannah honeybee (*Apis mellifera scutellata*). J. Invertebr. Pathol. 114 (1), 45–52.

Thrusfield, M., 2005. Veterinary Epidemiology, 3rd edn. Blackwell Publishing, England, pp. 345–543.

Tsuruda, J.M., Harris, J.W., Bourgeois, L., Danka, R.G., Hunt, G.J., Amdam, G.V., 2012. High-resolution linkage analyses to identify genes that influence varroa sensitive hygiene behavior in honey bees. PLoS One 7 (11), e48276.

Tulu, D., Aleme, M., Mengistu, G., Bogale, A., Bezabeh, A., Mendesil, E., 2020. Improved beekeeping technology in southwestern Ethiopia: focus on beekeepers' perception, adoption rate, and adoption determinants. Cogent. Food Agricult. 6 (1), 1814070.

Tulu, D., Aleme, M., Mengistu, G., Bogale, A., Bezabeh, A., Mendesil, E., 2023. Melissopalynological analysis and floral spectra of *Apis mellifera scutellata* Lepeletier bees in different agroecologies of Southwest Ethiopia. Heliyon. 9 (5), e16047 https://doi.org/10.1016/j.heliyon. 2023.e16047.

Underwood, R., Lopez-Uribe, M., 2022. Methods to control varroa mites: An integrated pest management approach. Penn State Extension (PSE), Pennsylvanian State University. ART-5874. Extension.psu.edu.

Wakgari, M., Yigezu, G., 2021. Honeybee keeping constraints and future prospects. Cogent. Food Agricult. 7, 1872192.