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MINIMIZ OF NOSEMA DISEASE INFECTION FOR HONEYBEE COLONIES BY USING SOME BEEKEEPING OPERATIONS IN MINIA REGION

Eshbah H. M.; A. R. Hassan; M. A. Atalla; H. M. Hassan and M. G. Darwish

Plant Prot. Dept. Faculty of Agriculture, Minia University

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ABASTRACT:

This study was carried out at two locations in Minia region, the apiary of Faculty of Agriculture Minia University and the other was private apiary at Shoushah village during 2014/2015 and 2015/2016 seasons. Also the study was subjected to four beekeeping operations i.e. reclaimed area, colonies with stable high population , colonies with renewing combs and colonies headed recent mated queens to reduce the mean percentage of nosema disease. Results indicated that the mean efficiency of the tested beekeeping operations could be arranged in the following descending order; colonies maintained in stable high population > reclaimed area > renewing combs > colonies having recent mated queens (31.39, 32.52, 35.24 and 37.52% mean nosema infection, respectively at season 2014/2015 also 30.94, 31.37, 34.20 and 37.82% mean nosema infection, respectively at season 2015/2016). Also data showed high significant differences of mean infection percentage by maintained colonies in stable high population and reclaimed area ($p= 0.0035$ and 0.0038), ($p= 0.00069$ and 0.0021) $p<0.01$ then renewing combs recorded significant difference ($p= 0.01138$, 0.01237) $0.05> p >0.01$.While colonies headed recent mated queens showed no significant difference ($p= 0.63872$, 0.20841) $p>0.05$ in 2014/2015 and 2015/2016 seasons.

Keywords: Nosema, beekeeping, reclaimed and combs

INTRODUCTION

Nosema disease is a parasitic disease of adult honey bees (*Apis mellifera* L.) causes by two species of microsporidia, *Nosema apis* Zander **(**Zander, 1909) and *Nosema ceranae* (Fries *et al.*, 1996). Transmission of nosema within honey bee colony is

mainly via the fecal-oral route in which pathogens are spread by transferring feces of diseased hosts to uninfected ones via ingestion. Adult bees ingest nosema spores when they feed on contaminated food and also when they are cleaning up fecal material from infected bees. The spores germinate within the midgut and release polar tubes that transfer their sporoplasm into midgut epithelial cells where they generate more spores. Millions of new spores can be found inside the midgut of bees within few weeks after initial infection **(**Bailey and Ball, 1991). Affected honey bees tend to die away from the hive, and there are no obvious signs of disease, which making it difficult to notice, hence it is often referred it as "the silent killer"(Hornitzky, 2005). Since late 2006, a mysterious illness, termed "Colony Collapse Disorder" (CCD), has been devastating massive numbers of honey bee colonies and threatens the beekeeping industry in the United States. As part of our efforts to identify the causes of sudden disappearances and extensive die-off of colonies in the hope of designing appropriate control strategies to safeguard bee health in the future, the status of nosema infections in honey bees collected from states affected by CCD was also examined.While our investigation did not show a direct correlation between nosema infection and CCD, the results revealed that *N. ceranae*, a species of nosema originally found in the Asian honey bee, *Apis cerana*e (Fries *et al.*, 1996).

MATERIALS AND METHODS

The present study was carried out in two locations of Minia region. The apiary of Faculty of Agriculture Minia University as cultivated area and the other was private apiary at Shoushah village as reclaimed area. The trails of the study were conducted through the two years of (2014/2015 and 2015/2016).

1. Investigation of bee samples:

Fifteen Carniolan honeybees colonies (*Apis mellifera carnica*), 9 colonies in each apiary. Colonies housed in Langstroth hives and having an approximately equal strength with bees covered 7 combs and equal stored food of honey and pollen were allocated to this study. Samples of adult honey bee workers were collected randomly from the entrance of different hives (Shimanuki and Knox, 2000). Each sample consisted of 100 adult honey bees collected from the hive entrance. (Vongpakorn and Neramitmansook 2003 and Sarlo *et al.*, 2011). Samples were collected around the month (Ingemar 1988). Crush adult bees with distilled water in a mortar (Topolska, and Hartwig, 2005 & Lotfi, *et al.*, 2009). Samples after preparation were examined by a light microscope for presence of Nosema spores. (Razmaraii *et al.*, 2013). Thereafter, the filtrate was examined microscopically at x 400 magnification, under-bright-field to determine the percentage of infected bees. (Topolska, and Hartwig, 2005). All bee samples were kept in 70% ethanol (Yanping, *et al.*, 2007) and

stored at –20 °C prior to examination (Gajger *et al.,* 2010).

Four beekeeping operations (reclaimed area, colonies with stable high population , colonies with renewing combs and colonies headed recent mated queens) were tested for avoidance of nosema disease as follows:-

Reclaimed area:-

Tree bee hives were placed on reclaimed area in Shoushah village and another tree hives placed on cultivated area in Faculty of Agriculture Minia University and having the same strength of bee colonies were left for normal conditions to act as control set. Transfer honeybee hives to dry location reduce the nosema infection compared with Mediterranean climate (Razmaraii and Karimi 2010)

Introduction of recent mated queens:-

Queens of three bee colonies were replaced by recent mated queens, while other three colonies were headed by two years old queens as control set **(**Zawilski and Skonieczna-Zawilska 1995).

Maintenance of the colony population:-

Three of the bee colonies were maintained in stable population (bees covered 9 combs) through addition of bees at necessary time to keep their strength in stable conditions, while the other three colonies were left to fluctuated population (5 to 8 combs covered with bees) which acted as control set (Gajger *et al.,* 2009).

Renewing combs:

Three of the bee colonies were subjected to replace old combs more than five years and another three hives having the same strength of bee colonies were left for normal conditions to act as control set (Fries 1991)

RESULTS AND DISCUSSION

Data in Tables (1 and 2) and Figs (1 and 2) for seasons (2014/2015) and (2015/2016) showed that the highest general mean percentages of nosema infection were recorded in the cultivated area (37.97% and 38.13%, respectively). While the general mean percentages of nosema infection in the reclaimed area record 32.52% and 31.37% infection, respectively.

Statistical and lyses showed high significant differences between mean infection percentage of infection in reclaimed and cultivated area ($p= 0.0038$ and 0.00215 respectively) $p<0.01$for the years of 2014/2015 and 2015/2016, respectively.

This may be due to the dryness of reclaimed areas comparing with the cultivated area. These results were agreement with Razmaraii and Karimi 2010 who found a significant relationship between the average number of spores per infected bee in the dryness area and rainy area.

Tables (1 and 2) and Figs. (1 and 2) reveal that the percentage infection values were higher in the cultivated area compared with that of the reclaimed area infection (37.97% and 32.52%, respectively). As for months data showed that the infection was the highest in Nov., Dec., Jan., Feb.,

March, April and May recording 30.89, 51.56, 91.67, 92.22, 74.78, 53.11 and 27.67%, respectively in the first season. However, the same trend was noticed in the second season.

Nosema activity was higher in cultivated area may be due to high levels of humidity which helps for more spore germination and long-time of living.

Data in Table (3) and Fig. (3) indicated that the mean efficiency of the tested beekeeping operations in reducing nosema infection could be arranged in the following descending order; using colonies maintained in stable high population > renewing combs > colonies having recent mated queens which resulted in 31.39%, 35.24% and 37.52% respectively.

Data showed that high significant difference of mean infection percentage by maintained colonies in stable high population ($p= 0.0035$) $p<0.01$ then renewing combs recorded significant difference ($p= 0.01138$) $0.05> p >0.01$.While having Colonies recent mated queens showed no significant difference ($p= 0.63872$) $p>0.05$.

Data in Table (4) and Figure (4) showed that high significant difference of mean infection percentage by maintained colonies in stable high population ($p= 0.00069$) $p<0.01$ then renewing combs recorded significant difference ($p= 0.01237$) $0.05> p >0.01$.While having Colonies recent mated queens showed no significant difference ($p= 0.20841$) $p>0.05$.

Also revealed that the mean efficiency of the tested beekeeping operations could be arranged in the following descending order; using colonies maintained in stable high population > renewing combs > colonies having recent mated queens which resulted in 30.94%, 34.20% and 37.82% respectively.

These results were agreed with that of Gajger *et al.,* 2009 who found that use of new wax, beekeepers devote insufficient attention or often neglect the disease and colony strength reduce the number of *Nosema ceranae* spores. Also (Botias et *al.,* 2012) show that adult honeybees caused by *Nosema apis* and *Nosema ceranae* is a common worldwide disease with negative impacts on colony strength and productivity. But (Findlay 2010) was against with this results which his results include: bee colonies headed by queens selected for their increased hygienic behavior exhibited reduced nosema spore loads.

Table (1): Monthly percent infection of honeybee by nosema disease in reclaimed area compared with cultivated area during 2014/2015 in Minia region.

Months	Reclaimed area				Cultivated area			
	Beginning of month	Middle of month	End of month	Mean % infection	Beginning of month	Middle of month	End of month	Mean % infection
May	25.00	22.67	20.00	22.56	29.33	28.00	25.67	27.67
June	4.00	2.33	3.00	3.11	9.00	5.00	5.33	6.44
July	2.67	1.33	1.00	1.67	4.33	4.00	3.00	3.78
Aug.	1.00	1.33	1.33	1.22	4.00	4.67	5.00	4.56
Sept.	3.00	4.00	4.67	3.89	4.33	5.33	6.00	5.22
Oct.	10.00	11.33	12.67	11.33	9.67	14.0	17.67	13.78
Nov.	18.00	25.67	28.33	24.00	22.67	34.00	36.00	30.89
Dec.	28.33	33.00	37.67	33.00	45.67	53.33	55.67	51.56
Jan.	80.00	80.00	79.00	79.67	89.33	91.67	94.00	91.67
Feb.	89.00	83.00	88.00	86.67	91.00	91.00	94.67	92.22
Mar.	72.00	70.33	67.00	69.78	77.33	74.00	73.00	74.78
April.	59.00	52.00	49.00	53.33	56.00	53.00	50.33	53.11
General mean			32.52			37.97		
T test Calculated					3.6459			
Probability (P)					*0.0038*			

Table (2): Monthly percent infection of honeybee by nosema disease in reclaimed area compared with cultivated area during 2015/2016 in Minia region.

Months	Reclaimed area				Cultivated area			
	Beginning of month	Middle of month	End of month	mean % infection	Beginning of month	Middle of month	End of month	mean % infection
May	16.67	17.00	20.33	18.00	26.67	28.33	29.33	28.11
June	2.00	2.00	3.00	2.33	5.00	3.33	3.00	3.78
July	1.00	1.33	2.67	1.67	2.00	1.67	1.67	1.78
Aug.	3.00	3.00	4.00	3.33	4.67	5.00	6.00	5.22
Sept.	2.33	2.33	4.00	2.89	4.33	5.67	6.0	5.33
Oct.	8.00	10.00	11.33	9.78	10.33	14.00	15.33	13.22
Nov.	23.33	24.00	25.00	24.11	26.67	28.00	30.67	28.45
Dec.	45.33	47.00	48.00	46.78	51.67	52.00	54.33	52.67
Jan.	79.33	79.00	80.00	79.44	89.00	91.00	92.33	90.78
Feb.	70.00	71.67	73.00	71.56	92.00	92.00	93.33	92.44
Mar.	70.00	67.33	69.00	68.78	80.00	78.33	75.33	77.89
April.	45.33	49.00	49.00	47.78	60.00	58.33	55.33	57.89
General mean			31.37			38.13		
T test Calculated					3.98005			
Probability (P)					*0.00215*			

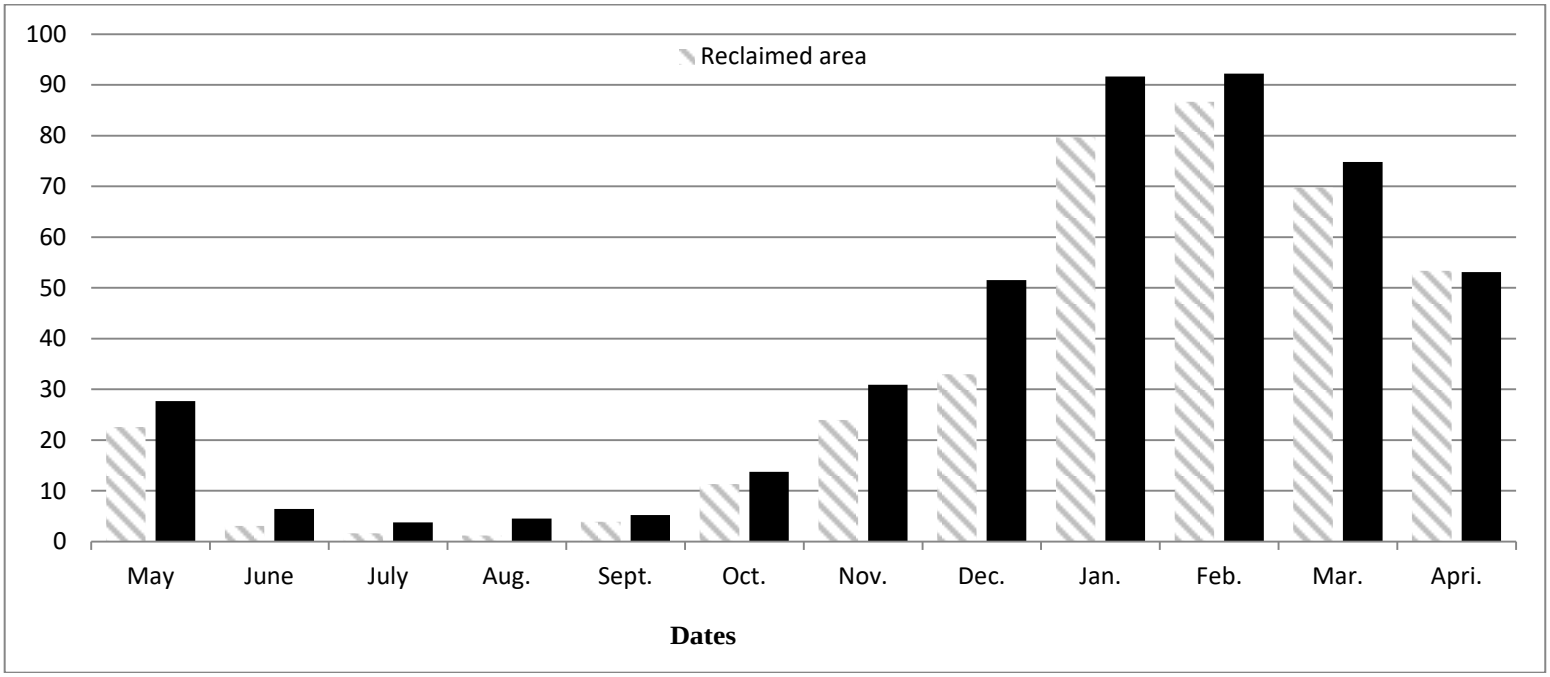

Figure (1): Monthly percent infection of honeybee by nosema disease in reclaimed area compared with cultivated area during 2014/2015 in Minia region.

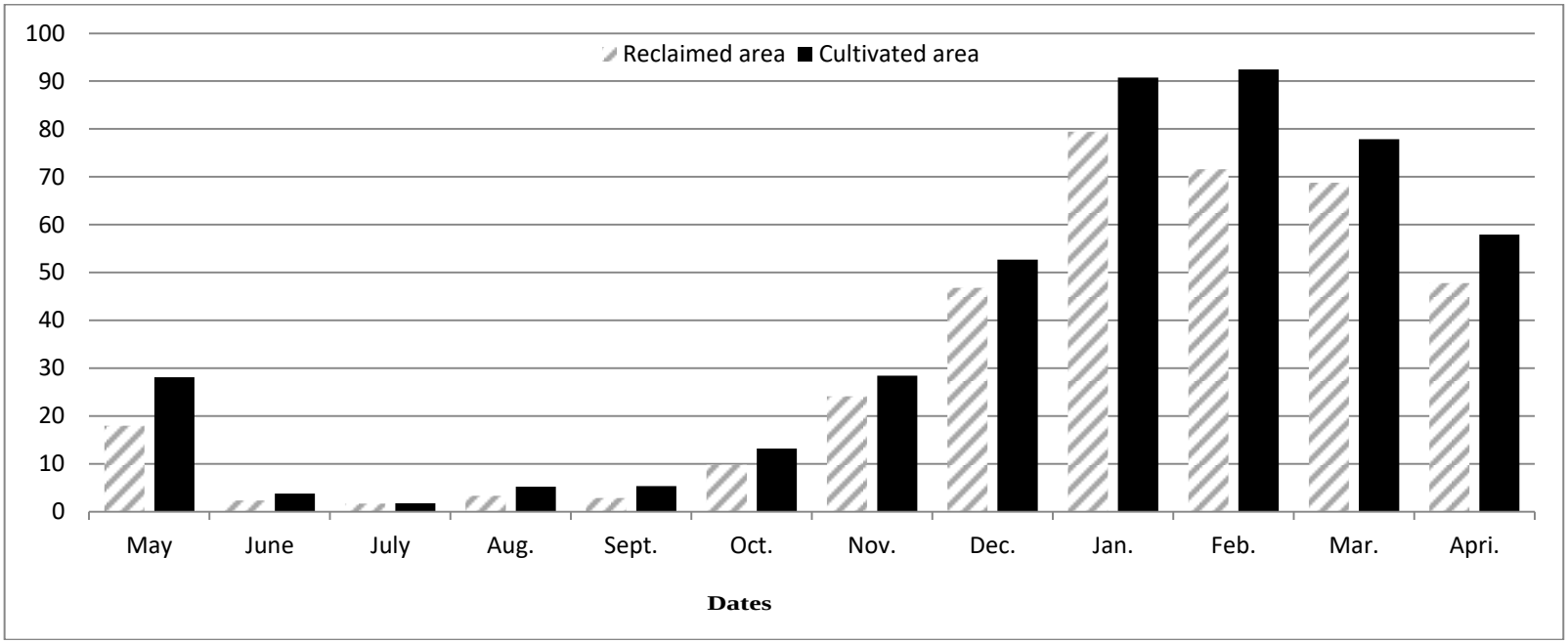

Figure (2): Monthly percent infection of honeybee by nosema disease in reclaimed area compared with cultivated area during 2015/2016 in Minia region.

Table (3): Effect of using certain beekeeping operations (Reclaimed region, Renew combs, Colonies headed recent mated queens and Colonies maintained in stable high population) in mean infection percentages of nosema disease during 2014/2015 in Minia region.

Month	Mean infection percentage in colonies with			
	Renewing combs	Recent mated queens	Stable high population	Control
May	25.78	27.22	21.55	27.67
June	3.89	4.89	3.78	6.44
July	2.11	3.67	3.56	3.78
Aug.	3.89	5.22	3.00	4.56
Sept.	3.33	5.00	2.78	5.22
Oct.	12.89	13.44	8.22	13.78
Nov.	30.78	31.55	24.67	30.89
Dec.	52.78	51.33	42.00	51.56
Jan.	81.00	94.00	80.33	91.67
Feb.	86.89	93.89	83.22	92.22
Mar.	71.11	68.44	61.67	74.78
Apri.	48.44	51.56	41.89	53.11
General mean	35.24	37.52	31.39	37.97
T test Calculated	3.03346	0.54211	5.08821	
Probability (P)	0.01138	0.63872	0.00035	

Table (4): Effect of using certain beekeeping operations (Reclaimed region, Renew combs, Colonies headed recent mated queens and Colonies maintained in stable high population) in mean infection percentages of nosema disease during 2015/2016 in Minia region.

Month	Mean infection percentage			
	Renewing combs	Recent mated queens	Stable high population	Control
May	26.56	30.22	22.22	28.11
June	3.55	3.67	3.33	3.78
July	1.33	2.11	3.55	1.78
Aug.	2.22	4.78	2.78	5.22
Sept.	2.85	5.00	2.67	5.33
Oct.	11.45	12.44	8.00	13.22
Nov.	30.44	30.33	23.78	28.45
Dec.	51.55	51.55	42.67	52.67
Jan.	81.67	90.89	78.67	90.78
Feb.	85.78	92.44	82.11	92.44
Mar.	67.11	71.56	60.44	77.89
Apri.	45.88	58.89	41.11	57.89
General mean	34.20	37.82	30.94	38.13
T test Calculated	2.9865	1.36863	4.66043	
Probability (P)	0.01237	0.20841	0.00069	

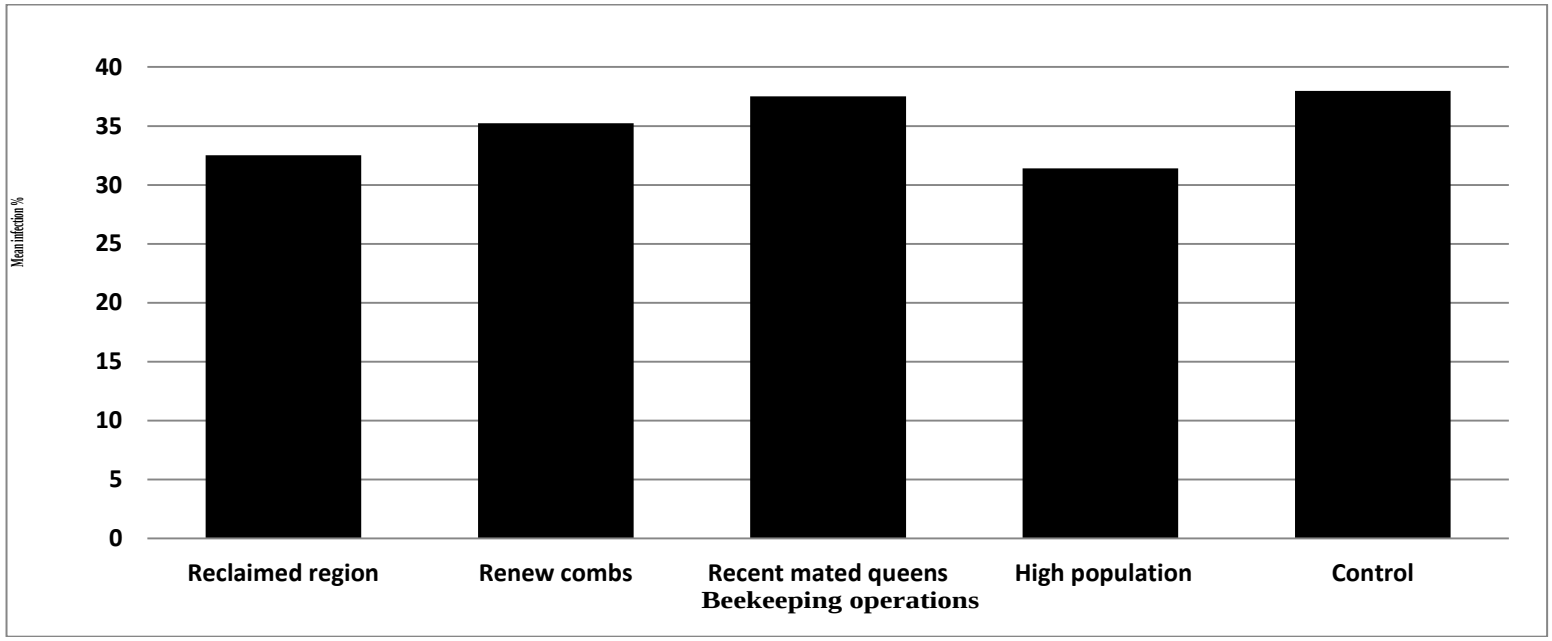

Figure (3): Effect of using certain beekeeping operations (Reclaimed region, Renew combs, Colonies headed recent mated queens and Colonies maintained in stable high population) in mean infection percentages of nosema disease during 2014/2015 in Minia region.

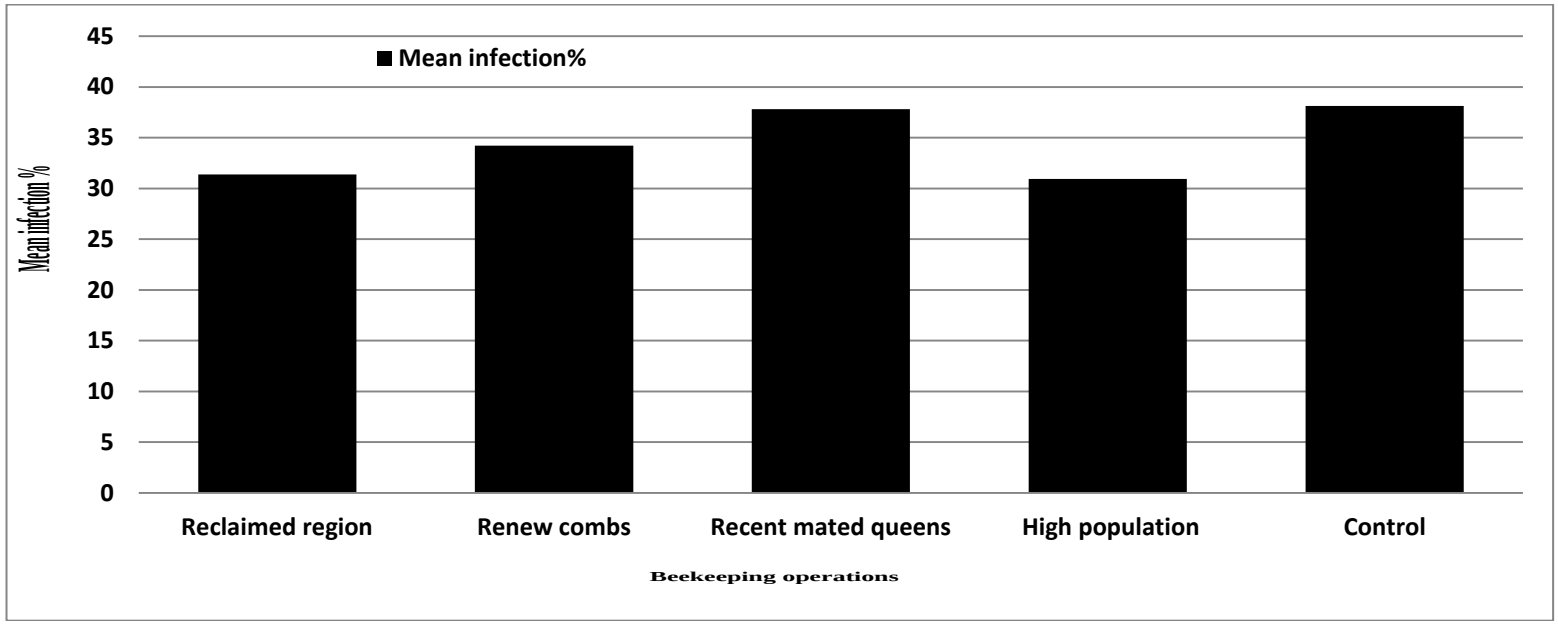

Figure (4): Effect of using certain beekeeping operations (Reclaimed region, Renew combs, Colonies headed recent mated queens and Colonies maintained in stable high population) in mean infection percentages of nosema disease during 2015/2016 in Minia region.

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تقليل الإصابة بمرض النوزيما لطوائف نحل العسل بإستخدام بعض العمليات النحلية في منطقة المنيا

حصافي محمد عشبة، عادل رشدي حسن، محمد ابو زيد عطا الله، حسن محمد حسن، محمود جمعة درويش

قسم وقاية النبات – كلية الزراعة – جامعة المنيا

تم إجراء هذه التجربة في منحلين بمنطقة المنيا المنحل الاول بكلية الزراعة والثاني بمنحل خاص بمنطقة شوشة على مدار موسمي 2015/2014 و 2016/2015. وقد تم تطبيق اربعة عمليات نحلية هي استخدام المناطق المستصلحة حديثا ، استخدام طوائف قوية ، تجديد الاقراص واخيرا استخدام طوائف ذات ملكات حديثة وذلك لتقليل نسبة الاصابة بمرض النوزيما. وقد اشارت النتائج الى انه يمكن ترتيب متوسطات كفاءة العمليات النحلية المختبره في خفض نسبة الاصابة تنازليا كما يلي اولا استخدام طوائف قوية ثم استخدام الاراضي المستصلحة حديثا ثم تجديد الاقراص واخيرا استخدام طوائف ذات ملكات حديثة (31.39 ، 32.52 ، 35.24 و 37.52% كمتوسط اصابة بالنموزيما على الترتيب لموسم 2015/2014م وايضا 30.94 ، 31.37 ، 34.20 و 37.82% كمتوسط اصابة بالنموزيما على الترتيب لموسم 2016/2015م). وايضا اوضحت النتائج وجود فروق عالية المعنوية باستخدام طوائف قوية و استخدام الاراضي المستصلحة (0.0035 و 0.0038) و (0.00069 و 0.0021 احتمالية) على الترتيب ثم يليها تجديد الاقراص حيث وجد فروق معنوية حيث ان الاحتمالية اكبر من 0.01 واقل من 0.05 (0.01138 و 0.01237 احتمالية). بينما استخدام ملكات حديثة لم يعطي اي فروق معنوية حيث ان الاحتمالية اكبر من 0.05 (0.63872 و 0.20841 احتمالية) وذلك للموسمين 2015/2014 و 2016/2015م على الترتيب.

الكلمات المفتاحية: نوزيما ، عمليات نحلية ، مستصلحة ، اقراص