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SURVEY OF RHINOCEROS BEETLE, *Oryctes* SPP. (COLEOPTERA: SCARABAEIDAE), IN ITS MICROBIAL IN DATE PALM ORCHARDS IN AL-MADINAH ALMUNWARAH REGION

Ibrahim, R. A. A.* and S. S. Alahmadi**

*** Economic Entomology Department, Faculty of Agriculture, Kafrelshiekh University, Egypt**

**** Department of Biology, Faculty of Science, Taibah University, Almadinah Almunawwara, P.O. 30002 , Saudi Arabia**

ABSTRACT

The Incidence and microbial natural enemies of *Oryctes* spp. (Coleoptera: Scarabaeidae) in date palm orchards in Al-madinah Almunwarah region, were studied. The different date palm cultivars can be arranged in descending order according to the infestation severity with *Oryctes* spp. as follow: Ajwah > Rothan > Safawi > Helwah > Shalabi > Barni and Rabbiah. The highest infestation rates of date palms with rhinoceros beetles were reached 44.1, 42.2 and 37.7% in September, August and November respectively. Whereas, the lowest infestation was recorded in February (10.2%) and January (11.7%). The population density decreased to reach the minimum values by mid January. The dominant stage of development occurred was the third larval instar. The first and second instars were rarely encountered. The vertical distribution of adults and immatures of the rhinoceros beetle around the heaps and root of the date palm was also studied. Data revealed that adults were more abundant in the surface layer at depth of 0:30 cm than immatures. While, immature stages were the most dominant at 30-60 cm depth. There were 72.3% of immatures (larvae and pupae) population found in the middle layer at depth (30:60 cm) around roots. In comparison, the third depth layer included only 5.3 % adults and 16.3% immatures. Four microbial natural enemies were recorded as mortality factors associated with the larval and pupal stages sampled within date palm orchards. The microbial analysis was narrowed down to detect only entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*), bacteria (*Bacillus* spp.), and virus (*Oryctes* virus). Entomopathogenic fungi caused average mortality to *Oryctes* larval population reached 15.3% and 9.2% by *Beauveria bassiana* and *Metarhizium anisopliae*, respectively. In comparison both entomopathogenic fungi caused 6.5% pupal mortality. On the other hand, *Bacillus* spp. caused the highest larval mortality reaching up to 39.2%, compared to 3.3% to pupae. Whereas, the percentage of infected larvae and pupae with virus recorded 27.6% and 11.5%, respectively and it was higher than of both pathogenic fungi (24.5%).

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a major crop in tropical and sub-tropical areas, with a yearly production of 5.4 million metric tons of fruit, mainly in the hot arid regions of South West Asia and North Africa. In addition to being the main component of oasis agroecosystems, it is used for food and as a building material. Progress in the field of crop improvement and expansion of commercial plantings has been mainly restricted by the habits and long-lived nature of this monocotyledonous tree, this being one of the main limitations of its mass production on a commercial scale. While seed propagation is impractical, offshoot production is impossible as it is limited to a certain period in the palm's life span and the limited number of offshoots produced (Ziad, 2002).

In the last few years, the Asiatic rhinoceros beetle, *Oryctes* spp. has emerged as a major pest of different date palm varieties (*Phoenix dactylifera)*. However, the adults are particularly troublesome during establishment of young palms and damage is expected to increase with the current extensive replanting schemes in Saudi Arabia, which provide an abundance of breeding sites for the pest as well as large numbers of susceptible young palms. The larval stage of this pest is long, several years in some species. The adults can attack many different palms including coconut, betel nut, sago palm and dates. They can also feed on pandanus and other fleshy plants (Vargo, 2000). The larvae feed on roots and rotten wood while the adults feed on nectar, plant sap and fruit. (Bedford 1986; Zelazny and Alfiler, 1991; Liew and Sulaiman 1993; Kamarudin *et al*., 2007; Soltani *et al.,* 2008).

Recently, three species of rhinoceros beetles were recorded in United Emirates. The most important and most widespread is *Oryctes agamemnon* which is a root-borer in its larval stage and a frond borer in the adult stage. In addition, two other species, *O. rhinoceros* and *O. elegans* which may be referred to as fruit stalk-borer; it can also act as a root-borers.

There are many control trials have been applied against rhinoceros beetles. For example, in Malaysia as an alternative in Palm Oil farms. There is a trend to use certain entomopathogens such as *Oryctes* virus to control this pest (Ramle *et al.,* 1999). It has been reported that, the introduction of the *Oryctes* virus to Pacific Islands led to a dramatic reduction in pest population and palm damage within 1–2 years. This virus infects both larvae and adults of rhinoceros beetle (Zelazny *et al*., 1992). Application of chemical insecticides such as carbofuran and cypermethrin has been widely used, but this method is not effective enough, costly and hazardous to non-target organisms (Chung *et al*., 1997; Dent 2000).

However, until now the ecological studies of *Oryctes* spp. in Arabic countries are rather limited. Therefore, this study aimed as a first step to determine the susceptibility of various date palm cultivars to infestation by the rhinoceros beetle, *Oryctes* spp. and survey of the associated microbial natural enemies with this insect pest in date palm orchards in Almadina almunawarah region.

MATERIALS AND METHODS

Date palm orchards

Infestation rates of seven local date palm cultivars namely, Rothana, Ajwah, Safawi, Shalaby, Helwah, Rabbiah and Barni with the rhinoceros beetle, *Oryctes* spp. were undertaken. Samples were collected from five date palm farms with area ranged from 5.4 to 8 hectares located in Almadinah suburban (85 to 100 km. north). The selected orchards comprised a mixture of date palm varieties in different ages and densities. There was no specific control management was adopted. Occasionally, carbofuran is applied mixing with soil around date palm trunk heaps and roots. In sever conditions;

workers are digging around the roots of date palm to collect larvae, pupae and adults.

Sampling technique

Sampling of insects was conducted from the date palm trunk heaps and roots. Sampled stages (larvae, pupae and adults) of *Oryctes* spp. were collected in sterile plastic containers and transferred to laboratory for further investigations. Twice a month, the five orchards were surveyed. Five palm trees of each cultivar were randomly chosen as samples to be examined for infestation. Moreover, the vertical distribution of adult beetles and immatures (larvae & pupae) around the date palm roots were also investigated.

Detection and characterization of microbial natural enemies

Both dead and alive stages of *Oryctes* spp. (larvae, pupae and adult) present in each sample were recorded and transferred to the laboratory. Each alive stage was reared individually in a plastic container (16 x 16 x 9 cm), half-filled with moist soil and shredded fragments of the palm roots. Each container was checked weekly for recording mortality and color change of larvae. In laboratory, dead ones were segregated. Infected cadavers were collected into individual sterile tubes until they were processed. The color and physical condition of the cadavers were characterized and paired with the results of the microbial analysis of the midgut. The detection of infesting fungi was carried out by plating samples from cadavers onto PDA- antibiotic Medium. Plates were incubated at 25±2˚C. The identification of developing fungal colonies and confirmation of its pathogenicity were carried out according to Leger (2008) and Reay *et al.*, (2008).

In case of hardened cadavers, the occurrences of white mycelium or green spores indicate the infection of *Beauveria bassiana* and *Metarhizium anisopliae,* respectively *(*Mohan and Pillai 1982; Sivapragasam and Tey 1995, Sharshir *et al*., 2006; Kamarudin *et al.,* 2007; Leger 2008; Reay *et al.*, 2008; Sun *et al*. 2008)). In addition, *Metarhizium* often caused brown, blister-like streaks on the cuticle of the larva before its death (Latch, 1976; Sivapragasam and Tey 1995). Similarly, a larva with a prolapsed rectum can also be classified as having infected with virus (Monty 1974; Bedford 1976, Ramle *et.al*., 2005). While for bacterial infection, each individual larva was dissected to remove its midgut. Occurrence and isolation of entomopathogenic bacteria were carried according to Thiery and Frachon (1997); Klein (1997); Xu-dong *et al*., (2007) Wehrle *et al.* (2010).Whereas, virus detection on cadavers was carried out according to Huger (2005); Ramle *et al*.(2005) and Prasad *et al*.(2008).

RESULTS AND DISCUSSION

Infestation of different date palm cultvars:

Table (1) shows infestation percentage of different date palm cultivars with *Oryctes* spp. The different cultivars can be arranged in descending order according to the infestation severity with *Oryctes* spp. as follow: Ajwah 50.1% > Rothan 41.9> Safawi 26.2%> Helwah 23.4%> Shalabi 18.7%> Barni 17.7% and Rabbiah 11.6%.

This results also revealed a significant increase in infestation rate in summer months. The highest average infestations of date palms with rhinoceros beetles reached 44.1, 42.2 and 37.7% in September, August and November, respectively. Whereas the lowest infestation was recorded in winter reaching 10.2% in February and 11.7% in January. The population density decreased to reach the minimum values by mid January at the tested localities. This fluctuation of date palm infestations from time to time may be due to the occurrence and/or absence and diversity of insect-pathogenic microorganisms in soil have focused on the differences in species composition between areas defined by habitat. Moreover, human activities and cultivation regimes influence the occurrence and abundance of soil-borne natural enemies of insects in agroecosystems (Klingen *et al*., 2002; Klingen and Haukeland, 2006).

The third larval instar was the dominant stage. The first and second instars were rarely encountered. This suggest the short duration of these life stages in the field. The higher percentage of beetles in date palm orchards, including some first instar larvae, may suggest some breeding activity in these orchards (Norman, 2004). The potential of *Oryctes* to generate subsequent generations might depend on environmental factors such as the decomposition stages of the trunk and thickness of the cover crop. The relatively long life cycle *Oryctes* (from 4 to 9 months) allows more than one generation per year. Therefore, the infestation incidence of date palm with *Oryctes* takes the whole year. The population of *Oryctes* in the trunks was drastically reduced; this could be due to the high percentage of mortality for the third instar larvae which caused a low survivorship for that period (Fig 4). These data are in accordance with that of Sivapragasam (2003) Soltani (2004) Kamarudin *et al*. (2007) Soltani *et al*. 2008. However, it became clear that the 3rd larval instar predominated throughout the year (excepting April and July). This is understandable since the 3rd larval is the longest developmental stage in the life history. Kamarudin *et al*.(2007) stated that the oviposition peaks occurred in February-April and September-October. Some palms contained a number of 3rd larval, and also eggs or 1st larval stage, indicating that different broods (at different developmental stages) were present. The rhinoceros beetle is very versatile in that it can use a wide variety of breeding sites of different date palm cultivars at the same time. For example, In coconut growing areas, decaying palm wood is important either in the form of dead standing poles, coconut trunks on the ground, or stumps. These beetles breed in organic manure pits and dung heaps; the tremendous increase in their numbers is mainly due to the mishandling of these manures in the farms – sometimes they develop on the palm itself, particularly the neglected uncleaned ones (Gassouma, 2004).

Thus plantation hygiene is very important in *Oryctes* infested areas (Samsudin *et al*.,(1993) Khoualdia and Rhouma, 1997). In date palm orchard ecosystem, the studies of population and infestation levels of rhinoceros beetles are fundamentally important to understand its relationship with the environmental factors and the role of its natural enemies. This approach means, it could be more equipped to control this pest effectively. Generally, ecological studies and the population fluctuation are important in management and control programs of numerous insect pests (Wood 1970, Dent 2000). Without this information, control techniques can be less effective, hence increasing the overall management costs. In south East Asia, there are many ecologically inclined approaches to assess the population of *O. rhinoceros* have been conducted on coconut (Hinckley 1973). However, many studies have suggested recently that in certain areas, *Oryctes* spp. can readily migrate to infest an area as soon as replanting is conducted, i.e. when there is an abundance of oil palm trunk chips (Bedford 1976; Gassouma 2004; Norman *et al.*, 2004).

Table 1: Differential susceptibility of different local date palm cultivars to *Oryctes* spp. infestation at Almadinah almunawarah region.

Month	% of date palm trees infested by Oryctes spp.							% infestation average
	Rothana	Ajwah	Safawi	Shalabi	Helwah	Rabbiah	Barni	
May2008	42.3	50.7	29.4	18.3	21.3	12.3	14.5	26.9
Jun.	48.7	56.3	33.7	21.5	29.8	14.2	19.1	31.9
Jul.	53	59.9	39.6	24.9	30	17.4	22.3	35.3
Aug.	62.1	78.4	42.7	27.9	34	21.4	28.7	42.2
Sep.	62	81.6	44.1	32.1	40.4	18	30.3	44.1
Oct.	63.9	70.3	30.3	32	29.4	13.4	24.4	37.7
Nov.	55.1	66.3	23.5	20.4	25.2	9.3	16.3	30.8
Dec.	31.9	51.1	11.4	9.2	14.4	8.4	13.4	19.9
Jan. 2009	12.4	19.2	9.2	9.5	11.1	7.3	13	11.7
Feb.	15.5	11.2	13.2	6.4	11.5	4.6	9.3	10.2
Mar.	21.2	18.7	16.2	10	17.2	4	9.6	13.8
Apr.	35	37.1	21.6	13.3	17.5	9.8	11.2	20.7
SE	5.35	6.86	3.54	2.64	2.72	1.56	2.1	
% average infestation	41.9	50.1	26.2	18.7	23.4	11.6	17.7	

Vertical distribution of different developmental stages of *Oryctes* in soil:

The vertical distribution of adults and immatures population of the rhinoceros beetle around the heaps and roots of the date palm was shown in Figure (1). Results revealed that adults are more abundant in the surface layer at depth of 0:30 cm than immatures. While, larvae and pupae were the most dominant at 30-60 cm depth. There were 72.3% of immatures (larvae and pupae) population found in the middle layer at depth (30:60 cm) around roots. In comparison, the third depth layer included only 5.3 % adults and 16.3% immatures. However, it was interesting to note that adult beetles can still search for breeding sites even under heavy ground cover (> 90 cm). However, the number of early instars found is almost negligible. On the other hand, majority of adults sampled were heavily occupying the surface layer which maybe to be available for mating and egg laying. These findings are in agreement with that of (Zelazny and Alwler 1986 and Zelazny and Alfiler, 1991) who reported that mating occurs in breeding sites of the upper surface. It is also expected, the environmental factors will influence the development, survival and distribution of adults and larvae onto the niche around date palm roots. It is difficult to evaluate the effects of temperature without considering moisture. Hinckley (1973) reported that moisture must be present to prevent desiccation and death of the developing immatures of *O. rhinoceros*. Moisture has an effect on the movement of the larvae. Several authors reported that larval migration into the soil as deep as 15 cm and as far as 40 cm from the center of the fecal pat. Soil type may have a major effect of the ability of larvae to migrate (Brown and Downhower 1988). The predilection of larvae to accumulate in the middle depth layer may due to be relatively close to debris, decaying roots and or optimum relative humidity content. It may due also to escaping from natural enemies and unfavorable environmental conditions.

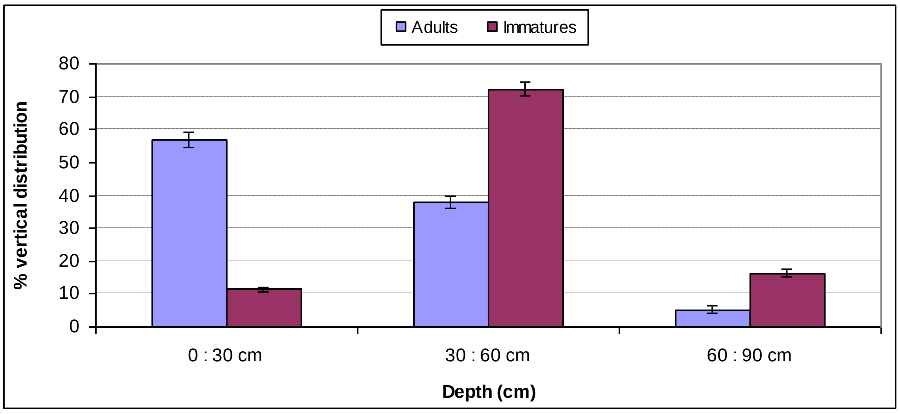

Figure 1: Vertical distribution of *Oryctes* spp. developmental stages.

Analysis of key mortality agents of *Oryctes* spp.

Figures (2 and 3) demonstrate percentages of total natural mortality recorded for larvae and pupae of *Oryctes* spp. Four microbial natural enemies were recorded. The mortality factors associated with the larval stages sampled within date palm orchards were firstly determined by visual symptoms with subsequent verification with microbial analysis. The analysis was narrowed down to detect only entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*), bacteria (*Bacillus* spp.), virus (*Oryctes* virus). Symptoms of mortality at the larval stages include soft bodied cadavers which were accompanied with a changing in color (Lacey and Brooks, 1997, El-Sufty and Boraei 1987; Soltani *et al.*, 2008). However, as shown in figure (4), there are also cases where infected larvae become hardened, followed by sporulations of the pathogenic fungus (Poinar and Thomas 1984, El-Sufty *et al*.,1993; Kamarudin *et al.*, 2007).

The entomopathogenic fungi:

Results indicated that both the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuilemin and *Metarhizium anisopliae* (Metsch.) are microbial natural enemies infecting and killing the larvae and pupae of *Oryctes* in their niches in date palm orchards. *B. bassiana* caused average mortality reached 15.3% of *Oryctes* larval population. Many authors have been reported that these fungi infecting different insect species belonging to Lepidoptera, Coleoptera and Homoptera (Schaerffenberg, 1959; Veen, 1968;

Fargues *et al.,* 1976, Sharshir *et al* 2006, Kamarudin *et al.* 2007, Reay, 2008). Generally, diseased larvae were sluggish and showed paralysis of the last abdominal segments. Cadavers of dead larvae were rigid and mummified with little dark brown spots on the pleural and sternal areas of the body wall. These fungi are very common in soil and there are more than 70 insect species have been recorded as its hosts (Steinhaus, 1949; Müller-Koegler, 1965). Historically, the preferred natural habitat of common entomopathogens such as *Metarhizium* and *Beauveria* has been uncertain. *Metarhizium* is most abundant in undisturbed pasture soils, 2–16 cm deep, where it can reach 106 propagules/g. It has not been clear whether the propagules recovered are conidia, mycelia surviving on insect remains, or mycelia living on non-insect substrates as saprophytes (Milner, 1992). Sun *et al*. (2008) surveyed a total of 20 species of insect-associated fungi in orchard soils. The survey included insect-pathogenic fungi and opportunistic insect pathogens. They recorded three insect-pathogenic species, *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces fumosoroseus* (Ascomycota: Hypocreales). However both *B. bassiana* and *P. fumosoroseus* were more frequently detected in orchard soils, than *M. anisopliae.*

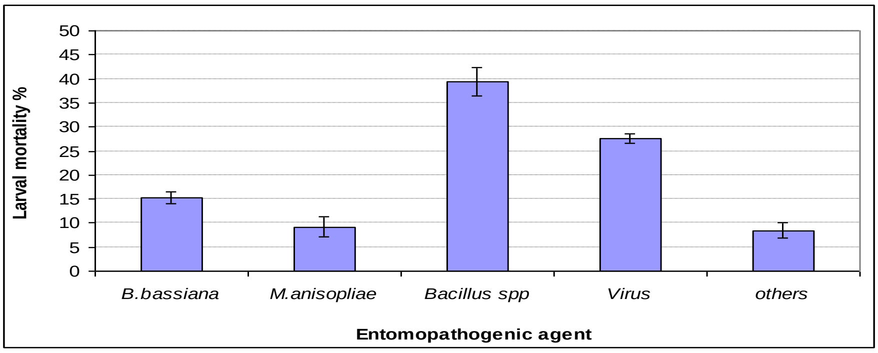

Figure 2: Mortality agents of *Oryctes* spp. larvae.

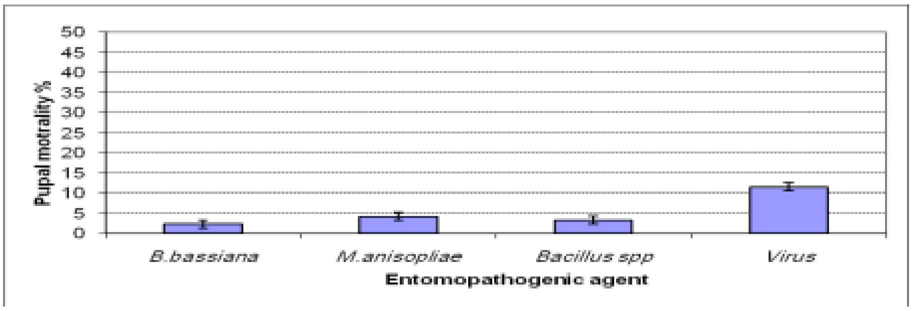

Figure 3: Mortality agents of *Oryctes* spp. pupae.

In case of infected *Oryctes* larvae and pupae with *B. bassiana*, cadavers were rigid and mummified with little dark brown spots on the cuticle. Some of them were found partially or completely covered with flat white mycelial growth of the fungus. These larvae became rigid and mummified within 3-4 days at room temperature. The fungus mycelia grew on the rigid cadavers covering its entire surface bearing mealy white conidia (Fig. 4 b). Most previous studies concerning the occurrence and diversity of insect-pathogenic fungi in soil have focused on the differences in species composition between areas defined by habitat types (e.g., cultivated soils, natural soils, etc.) at one point in time. However, Meyling and Eilenberg (2006) compared the occurrence and abundance of insect-pathogenic fungi between two consecutive years in a single organically farmed field and found that high and low densities of the fungi occurred within specific areas.

While, infected larvae with *M. anisoplia,* mycosed larvae found in the field were feeble with large irregular yellowish brown areas on the cuticle (Tey, 1993). The larval cadavers were rigid and covered with mycelia growth like copper-rust in color, so it is commonly referred to as the green muscardine fungus as shown in Figure (4 a). Smears of haemolymph and fat bodies of alive diseased larvae indicated the presence of short branched hyphae with dense cytoplasm and blastospores. *M. anisoplia* caused average mortality reached 9.2% of *Oryctes* larval population (Fig. 4a).While both *Beauveria* and *Metarhizium* caused pupal mortality reached 2.3 and 4.2% respectively (Fig.3). Mortality, symptoms and pathogenicity of *M. anisopliae* to *Oryctes* spp. larvae are similar with that described for some lepidopterous and coleopterous larvae infected with *M. anisopliae* (Weiser, 1969; Zimmermann, 1992 and Sewify and Sharaf El-Din, 1993, Kamarudin *et al.*, 2008). Generally, most of diseased larvae were during the 3^{th} larval instar, while some larvae were still alive after infection and able to molt and reaching the pupal stage.

The entomopathogenic bacteria

Results of the microbial analysis indicated that the soft, flaccid and black cadavers recorded 39.2% and 3.3% of *Bacillus* spp. for larvae and pupae, respectively (Figures 2 and 3). Microbial analysis showed that there were no occurrences of either virus or fungus on the shrunken and brown cadavers. This may suggest that the bacteria may be solely responsible for those symptoms. These results are in agreement with that of Kannan *et al.*, 1980. However, in soft and brown cadavers, the percentage of bacterial occurrence was higher than virus or fungi (Figure 4c). Bedford (1980), reported that the third larval instar had the longest developmental period (between 60-165 days), which exposed the larvae to various diseases. Therefore, the timing of application of any biocontrol agent can be targeted to the third larval instars for an effective control (Babu *et al.*, 1971; Hochberg and Waage 1991).

The entomopathogenic virus

Microbial analysis revealed that, on soft and pale larval cadavers, the percentage of having virus infection recorded 27.6% was higher than both pathogenic fungi (24.5%) and less than bacteria. The virus caused pupae mortality reached 11.5%. Larvae and pupae cadavers were soft, fragile and ruptured when touched; releasing liquefied whitish green contents (Fig. 4 c). About 24 hour after death, larval cadavers became partially or completely yellowish brown (pale) in color due to putrefaction processes caused by saprophytic bacteria. It has also noted that, adult beetles have been reported as vectors of virus and fungus (Monty 1974; Zelazny *et al*., 1992; Ho 1996). Based from external symptoms, the developing stages could have been infected by virus and bacteria. All replanting orchards in this study have indicated low mortality of the first and second larval instars which might be due to viral or bacterial infections. Besides *Oryctes* virus, there may be other groups of viruses involved (Seguni *et al.*, 1999; Kamarudin *et al*., 2007). Moreover, based on microbial analysis, there was a higher probability (8.4%) of the larvae dying from unknown causes (could be due to low nutritional conditions or inability of the larva to accumulate enough fat reserves for pupation). Other mortality factors involved could be naturally induced, either due to nutrition, physiological or mechanical stress. Hinckley (1973) reported that mortality percentage of the larval stage was high at the first instars, when they start feeding on hard substrates, and at the third instars, when they could not accumulate enough fat reserves to enter the pupal stage.

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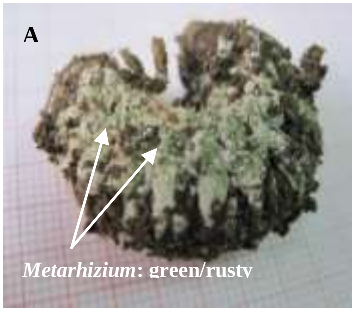

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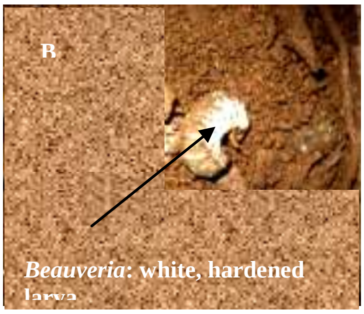

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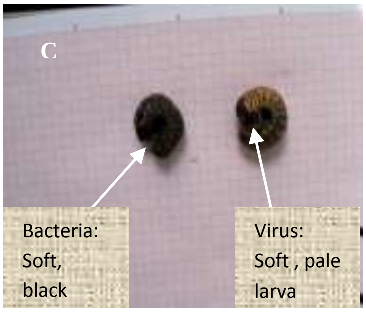

Figure 4: Morphological signs and symptoms of infected *Oryctes* larvae with different bioagents.

In conclusion, based on the external symptoms and microbial verifications, the majority of the larval stages may have died due to bacterial and virus infections. The first and second larval instars may have died mainly due to virus. The third instars and pupal stages may have been infected by bacteria. mortality Percentage of the larvae was always higher in the third instars compared to other instars. The first instar recorded the lowest mortality. Infection of *Metarhizium* at the larval stages was less than that of Beauveria (Babu *et al., 1993)*. Moreover, based on microbial analysis, there was a higher probability of the larvae dying from unknown causes (could be due to low nutritional conditions or inability of the larva to accumulate enough fat reserves for pupation). Ecological studies revealed that without any control measures, *Oryctes* population has been found to establish itself in the date palm trunk heaps, up to a period of about two years. Knowledge of the species composition and distribution of indigenous insect pathogens is essential for assessing the biocontrol potential in the agroecosystem. To prevent economic losses, (1) it is necessary to identify and remove or destroy these beetle-producing sites. (2) collection and destruction of adults and immature stages of the beetle. (3) incorporation of the entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae, Bacillus* spp. and the *Oryctes* virus in its breeding sites to suppress the perpetuation of the pest (Liau and Ahmad, 1991).

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حصر لخنفساء وحيد القرن من جنس أوركتس وأعدئها الطبيعية الميكروبيه في بساتين نخيل التمر في منطقة المدينة المنورة.

رضا عبد المنصف أحمد ابراهيم* و سعيد سعيد الأحمدي**
*** قسم الحشرات الاقتصادية – كلية الزراعه – جامعة كفرالشيخ - مصر**
****قسم البيولوجي – كلية العلوم – جامعة طيبة – المدينة المنورة، ص ب: 30002 ،المملكة العربية السعودية**

تعتبر خنفساء وحيد القرن *Oryctes* spp. (Coleoptera: Scarabaeidae) من الآفات الخطيرة في الأونة الأخيرة والتي تصيب بشدة جذور الأنواع المختلفة لنخيل التمر في منطقة المدينة المنورة. تناولت هذه الدراسة حصر الأعداء الطبيعية الميكروبية لهذه الآفة وكذلك تم تقدير مدي شدة اصابة الأنواع المختلفة من نخيل التمربها. وأوضحت الدراسة أنه يمكن ترتيب الأنواع وفقا لشدة اصابتها كمايلي: العجوة، الروثان، الصفاوي، الحلوة، الشلبي، البرني وأخيرا الربيعة. وقد سجلت أعلى نسب اصابة بهذه ألافة وبلغت 44.1، 42.2 و37.7% في سبتمبر، وأغسطس ونوفمبر على التوالي، بينما بلغت أدنى مستوياتها في فبراير ويناير مسجلة 10.2 و11.7% على التوالى. الطور الأكثر تواجدا في العينات كان العمر اليرقي الثالث ، بينما كان تواجد العمرين الأول والثاني نادرا. كذلك تمت دراسة التوزيع الرأسي لهذه الآفة وأطوارها المختلفة في منطقة الجذور حيث أوضحت النتائج أن الحشرات الكاملة تكون سائدة في الطبقة السطحية للتربة (على عمق من صفر – 30سم) ، بينما تكون الأطوار غير الكاملة سائدة في على عمق من 60 – 90 سم حول منطقة الجذور.على الجانب الآخر فان تواجد الخنافس الكاملة والأطوار غير الكاملة كان نادرا على أعماق أكثر من 60 سم. وجدير بالذكر أنه تم تسجيل أربعة أعداء طبيعية ميكروبية ليرقات وعذارى هذه الآفة. فقد تم تسجيل نوعين من الفطريات الممرضة وهما البوفيريا والميتاريزيم حيث سجلا نسب موت بلغت 15.3% و9.2% لليرقات على التوالى بينما كانت هذه النسبه منخفضة في حالة طور العذراء وبلغت 6.5% للأثنين معا. كذلك تم تسجيل بكتيريا الباسيلس تصيب اليرقات والعذارى بنسب بلغت 39.2%، و3.3% على التوالي. كذلك تم تسجيل أحد أنواع الفيروسات حيث بلغت نسبة اصابة اليرقات بة 27.5% بينما نسبة اصابة العذاري بلغت 11.2%.

قام بتحكيم البحث

أ.د / عادل حسن عبد السلام	**كلية الزراعة – جامعة المنصورة**
أ.د / ابراهيم ابراهيم مصباح	**كلية الزراعة – جامعة طنطا**