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Comparison Of Extraction Efficiency Between Extraction Box of Birds Ectoparasite (E.B.B.E*) and Tullgren Extractors for Ecological Parasitology Studies

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Abstract: Comparison of Extraction Efficiency between Extraction Box of Birds Ectoparasite* and Tullgren Extractors for ecological surveys studies of macroparasite such as ticks (*Argas streptopelia*), fly (*Pseudolynchia canariensis*) and two species from lice (*Columbicola columbae* and *Goniocotes gallinae)* were collected from wild laughing dove. Concerning the microparasite, there were *Ornithocheyletiadubinini* which associated with *Streptopelia senegalensis* and *Dermoglyphus columbae* and *Hemimyialges* sp.. These two devices differ in their extraction efficiency. In this study, we investigated the extraction efficiency of Extraction Box of Birds Ectoparasit and Tullgren under the same conditions in both types of devices. Extraction Box of Birds Ectoparasite extraction was more effective for extracting tick, mites,lice and fly than Tullgren extraction both qualitatively and quantitatively. The number of specimens collected by Extraction Box of Birds Ectoparasite* extraction was consistently higher than Tullgren extraction for all the samples macroparasiteand microparasite, this is due to the presence of the bird in an area that allows him to move, in addition to benefiting from the movement of bird feather's, which had accompanied the fall of parts of feathers, which may be infected with some species o facarine which parasites on the feathers. Qualitatively, Extraction Box of Birds Ectoparasite extraction yielded more micoparasites than Tullgren extraction. Thus for more complete species inventories in ecological and taxonomic studies, Extraction Box of Birds Ectoparasite extraction method is more effective than Tullgren.

Key words: New method · Isolate · External parasites · Ectoparasite · Macroparasite · Microparasiteand Tullgren funnels

INTRODUCTION

To study arthropoda such as tick,mites and lice, special techniques are needed to extracted from the animals, soil and litter. Many specialized extractors have been developed to assess animal diversity in soil and litter including Tullgren (Berlese) funnels(high-gradient funnels) and Extraction Box of Birds Ectoparasite [1,2]. The principle mechanism of the extraction is that the funnel creates warm and dry condition at the upper part by a lighting source equiped on the top, which leads the litter and soil dwelling invertebrates to move down the funnel away from the light source and finally fall out to collecting bottle [1,3]. Different authors have argued about the efficiency of this extraction devices, e.g. Kalif and Moutinho [4] and Longino *et al*. [5]. Aim of this study, Comparison of Extraction Efficiency between Extraction Box of Birds Ectoparasite (E.B.B.E*) and Tullgren Extractors for ecological surveys studies of macroparasite and microparasiteof infested wild laughing dove*Streptopelia senegalensis* in different seasons and localities at Taif Governorate with Acarine from 2010 to 2011.

MATERIALS AND METHODS

Collection of Wild Birds Samples:

Bird Samples Were Collected by Mist-Nets: A dawn-to-dusk mist- netting was conducted with the aim of capturing as many bird species and individuals, as possible and to examine them for the presence of ectoparasites. The length of the mist-nets was ~30 meters and the high was ~ 5 meters, mist-nets were linked in many places in Taif (KSA). Mist-nets was checked at least twice days, noted every individual bird was identified [6]. The right and left sides of the

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mist-nets were supported with two wood stand, which linked to any near trees or electricity poles in the area. Fig. (1).

Collection of Arthropoda Samples: Each bird was examined manually under the stereomicroscope for any infestation on the beak or legs and in different parts of the body especially ventral part between the sternum and cloaca and below the wings [7], Fig. (2-4).

Collection by Tullgren'sfunnels: The feathers were removed especially from the abdominal area and sometimes takes the total feather of the individuals that have been slaughtered and placed inside the Tullgren's funnels. Fig (5-6) .

Feather was put inside the Tullgren's funnels after remove the drip filter of the extracting unit, then a small dish filled with water or wet cotton was put under each funnel of the unit. Samples were receiving in dishes which contain wet cotton or water. Feathers were leaved in the Tullgren's funnels for a period not less than (12) hours, after (6) hours at least extracted dishes were examined under the stereomicroscope. In the presence of acarine, samples were isolated by camel brush and mounted in Hoyer's medium for identified by light microscope [8].

Collection by Extraction Box of Birds Ectoparasite*: The isolation was done by a new device know Extraction Box of Birds Ectoparasite (E.B.B.E)*, which was invented by the researcher.

Device Design (E.B.B.E*): Device was invented by the researcher Wafa Mohammed Al-Otaibi and she obtained a patent certificate number 3296 in 11.02.2014 issued by the King Abdu laziz City for Science and Technology - kingdom of Saudi Arabia

It was a box provided with opening for ventilation to prevent the bird's death by suffocation during the examination period, after receive the arthropoda samples which isolated from the bird on folding material put in a bottom drag drawer, to ensure that the isolated samples never escape out during the drawer and dump,.its can surround the edges of the drawer with adhesive as Vaseline. Fig (7). After one hour, collected the contents of the bottom drawer and examined under a microscope, Fig. (8). The model of Extraction Box of Birds Ectoparasite extractors used in this study for extracting macro and micro parasites from a live birds and Mamalis.

Model of Tullgren Extractor: The model of Tullgren extractors used in this study was made of tinplate steel and consisted of three removable parts: funnel shaped cover, main canister and funnel-shaped bottom. The dimensions of each part are (diameter ×height), 38×17 cm, 38×26 cm and 38×17 cm respectively. Inside the main canister is a 34×17 cm (diameter ×height) internal stainless steel basket (with cover of 1×1 cm wire mesh net) placed in its main part. All removable parts were well tightfitting.

The model ofinvention Extraction Box of Birds Ectoparasite which showed in Fig (9), it is a box with high (35 cm) and width (45 cm), made from aluminum [the back side (7),upper side (1) and bottom sides (12)], while the right (4), left (8) and the front sides (11) are made of glass. The back side (7)contains three slots (3-b) (3-c) (3-d) its diameter is (2.2 cm) for ventilation. Fig. (10) show the back side (7) and the front side (11) contains two pieces of metal on each side in two levels (9-a) (9-b) to install a mesh net to appreciate appropriate to the size of the bird. Where the higher high (9-a) is the closest to the electric lamp in case of small size birds such as sparrows, whereas the less high (9-b) and closest to the bottom drawer in case of the big size birds, such as the pigeon.The upper side (1) contains internal two electricians lamps (2) 220 volts and circular opening 5 cm in diameter (3-a) for ventilation The upper part represents the cover of box closed by special clip (13) to tightly closed.

Fig. (11) show the right side (4) which contains two separate rectangular opining ((5-a) (5-b) extends along the width of the device, its long (30 cm) and width (2.2) cm and the distance between them (7) cm. Each of them have aluminum cover (6-a) (6-b) and these openings use to enter the mesh (10) with length (43.5 cm) and width (30 cm) for allowing the birds to stand. At the top of the cover behind the electric light (2) there is a switch (14) for electric light supply.While the bottom of box, is a drawer drag (12) and its dimensions is (30. × 38 cm).Folding material put in a bottom drag drawer and dump its contents in a dish for examination.

Samples and Extraction Procedures in Tullgren Extractor: Ten samples were loaded into a single mesh net basket and placed into a Tullgren extractor. Each Tullgren extractor had a 60W incandescent light positioned above the soil sample. The 60 W incandescent light was turned on throughout the extraction period. A collecting bottle containing 80% ethanol was placed under each Tullgren extractor to collect the falling arthropods.

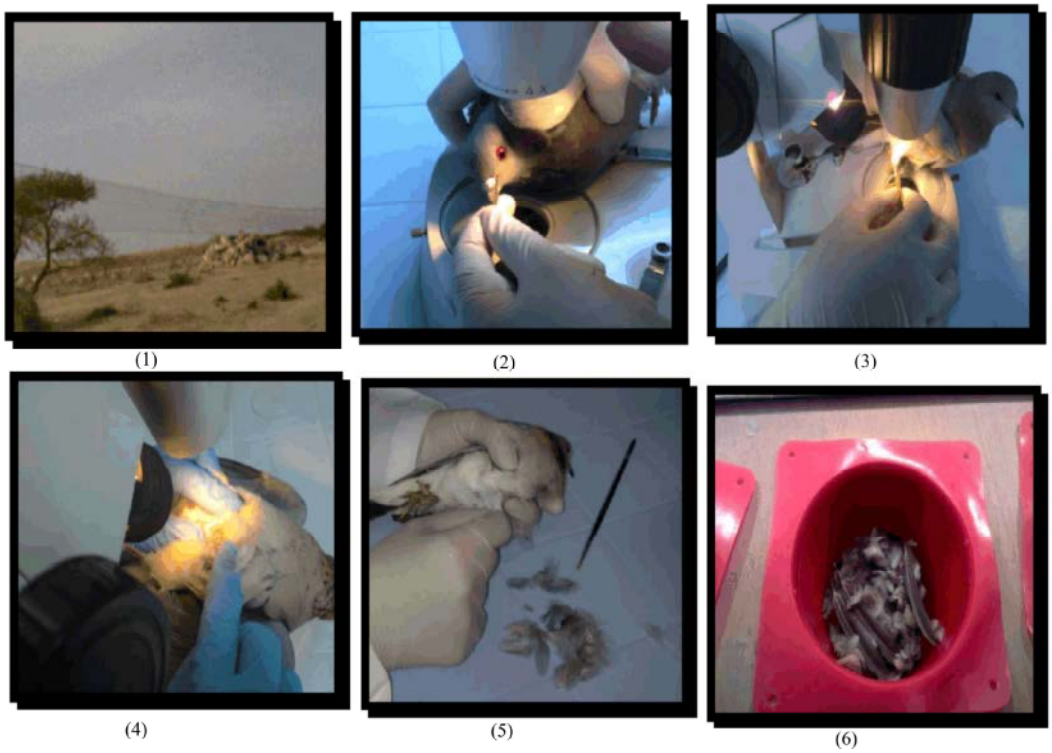

Fig. 1: mist-nets
Fig. 2: Examination the beak
Fig. 3: Examination the legs
Fig. 4: Examination the ventral body
Fig. 5: Remove the feathers of the bird
Fig. 6: The feathers inside funnels

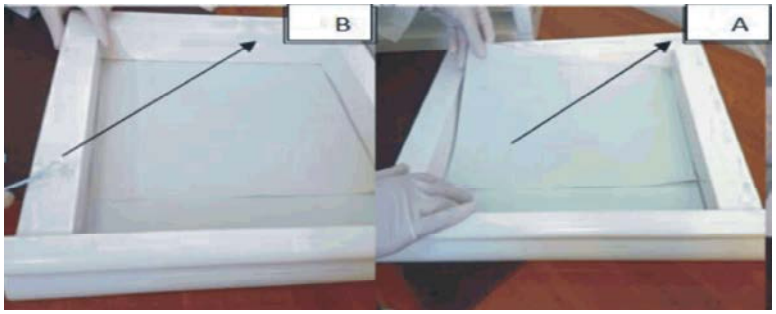

Fig. 7: A) Folding material in a bottom drag drawer B) Surround the edges of the drawer by adhesive

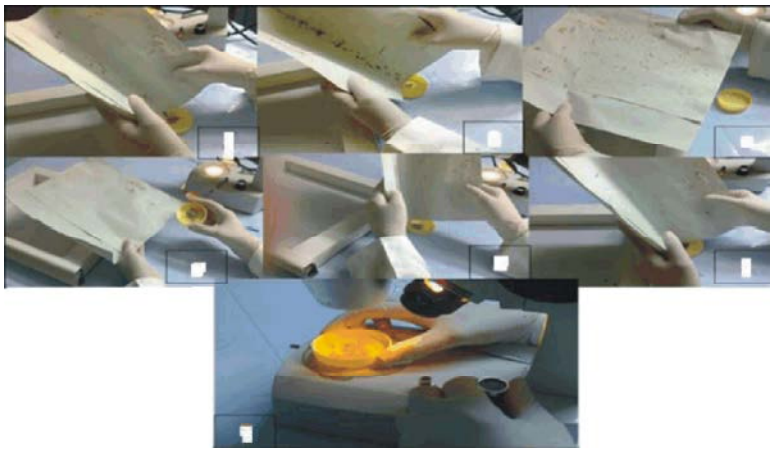

Fig. 8: Dump the drawer

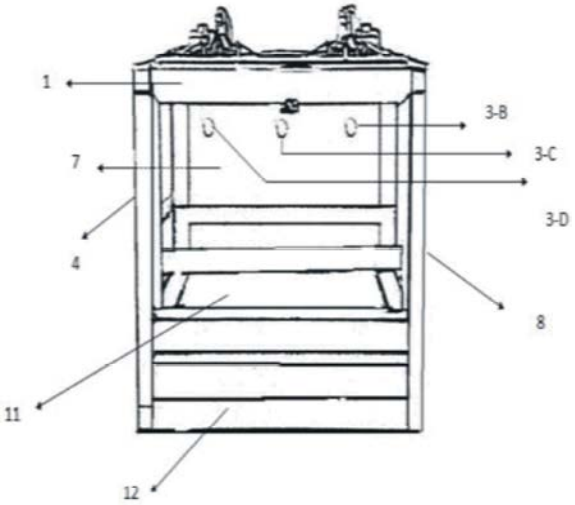

Fig. 9: General view of thedevice

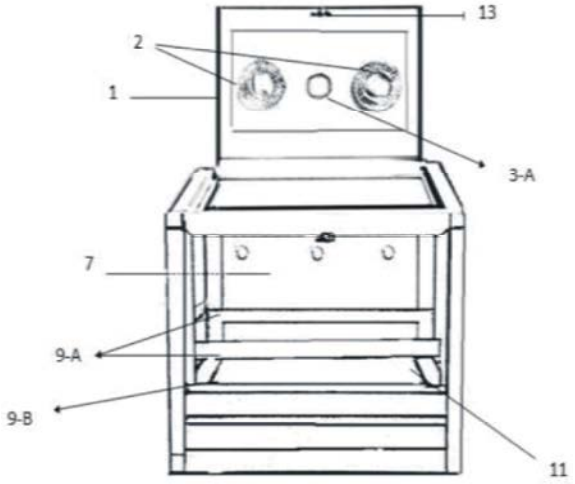

Fig. 10: Fornt view and thedevice is open

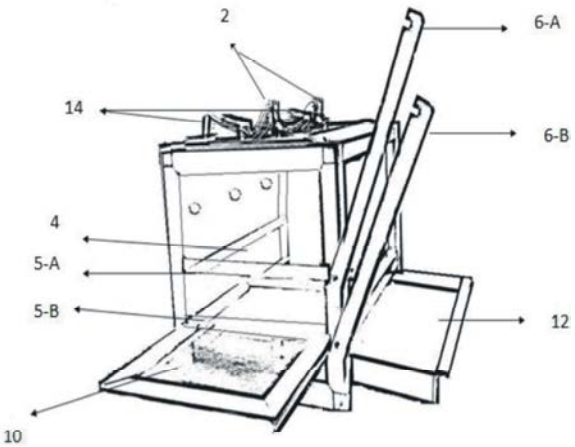

Fig. 11: lateral view of the device

Samples and Extraction Procedures in Extraction Box of Birds Ectoparasitethe method of sampling is, by put folding material in a bottom drag drawer and put Vaseline on the edges of the drawer to ensure the isolated samples never escape out the box, then determining the appropriate height of the mesh net by size of the bird, then put the live bird inside the device and keep the electric light on then close the box. Post one hour, dump the contents of the bottom drawer for microscope examination. Re-folding material in the drawer and complete the examination, finally release the bird [8].

Target Groups: All arthropda samples extracted with Extraction Box of Birds Ectoparasite and Tullgren extractors were counted and identified according to Gille [10]. Comparisons of extraction efficiency were done for the two extractors.

Data Analysis: The data which were recorded during the study period were entered into Microsoft excel sheet. Data were summarized and analyzed using SPSS version 16 computer program. Data were analyzed using Epi Info version 6 statistical software [9] and for further compared using Chi-square test at critical probability of $p<0.05$.

RESULTS AND DISCUSSION

A total number of collected laughing dove *Streptopelia senegalensis* Linnaeus from different places and during the four different seasons in Taif - Kingdom of Saudi Arabia wild was (110). This species belonging to famil (Columbidae), as recorded by Gille [10].

The results showed 24 out 110 wild laughing dove was infested by one species of ticks, three species of mites, two species of lice and one species of flies Fig. (12). The wild *Streptopelia senegalensis* individuals were captured and examined from different localities. In mountain (32) birds were examined eight of them were infested. In plains (12) individuals were infested and (36) individuals were uninfested. While in farms and residential two birds were infested, (15) and five birds were uninfested, respectively. Six individuals from valley were examined and they were uninfected as showing in Table (1). Howeven, the statistical analysis showed that there was a significant different between the infested and uninfested laughing dove in different habitats ($t= 3.33$, $P<0.05$).

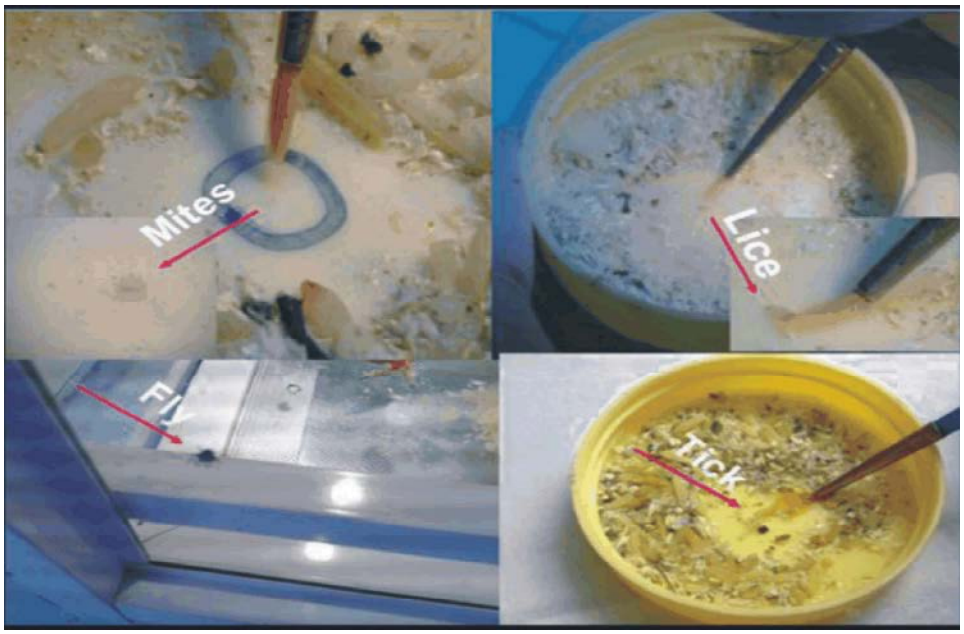

Fig. 12: Different species of ectoparasites which isolated by the device Extraction Box of Birds Ectoparasite

Table 1: The number and species of infested wild laughing dove in different localities at Taif Governorate with acarine species:

		Extraction method			
Species of bird	Types of localities	Species of acarine	E.B.B.E*	Tullgren	Number of birds
Streptopelia senegalensis	Mountain	*Argas streptopelia- Ornithocheyletiadubinini*	8	2	32
	Plains	*Argas streptopelia- Dermoglyphus columbae*	12	3	48
	Farms	*Argas streptopelia*	2	1	17
	Valleys	----	0	0	6
	Residence	*Argas streptopelia- Ornithocheyletiadubinini*	2	0	7
	Total number of Ectoparasite		24	6	30
Total number of birds					110

These results were coincided with the results of (11 and 12), as showed in Table (2) *Streptopelia senegalensis* were examined in different seasons, 24 out 110 individuals were infested. In Winter and Spring there were one and two infestation, respectively. Whereas in Summer, eight birds were infested In Autumn (13) individuals were infested.This results showed that there was highly significant different between the infested and uninfested laughing dove in different seasons ($t= 6.44$, $P<0.001$), these results were strongly supported by Sakchoowong *et al* [11] and Chandler [12].

Concerning the species of acarine among infested wild laughing dove in different localities at Taif Governorate (Table 1), revealed that *Streptopelia senegalensis* specimens from different localities were infested with three various species of acarine, one of them belonging to ticks and the remaining species were belonging to mites. In mountain and residential there were two species, the first one was *Ornithocheyletiadubinini* (Cheyletide: Prostigmat) Fig. (13). The second species was *Argas streptopelia* (Argasidae: Metastigmata) Fig. (14) and their larval stage. In the plains there was different mites, in addition to *Argas streptopelia*, *Dermoglyphus Columba* (Dermoglyphidae: Astigmata) Fig. (15). Whereas in farms the infestation was only with *Argas streptopelia*, while in valleys the examined birds were uninfested.

Fig. 13: External morphology of male of Ornithocheyletia dubinini by light

Fig. 14: Dorsal view of larva of Argas streptopelia by light

Fig. 15: Antero ventral of Dermoglyphus columbae by

The highest infestation was in residential with ratio (28.5%), while the ratio in mountain and plains was equal (25%), whereas in farms the infestation ratio was (11.7%). In valleys it was absent.In the current study, results is in strongly agreement with the results of CHUNG, *et al*. (2000), [13].

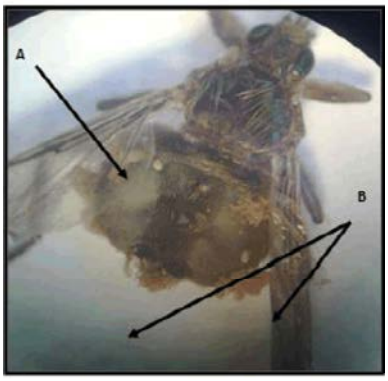

Fig. 16: Dorsal view of infested louse fly with Hemimyialges sp. by light microscope A) The female of Hemimyialges sp. on the B) Clusters of eggs of Hemimyialges sp.

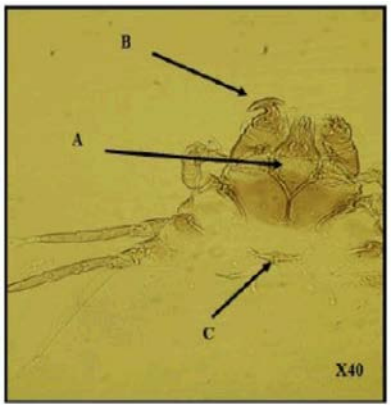

Fig. 17: The proterosoma of Hemimyialges sp. by light microscope A) Gnathosoma B) Claw C) Genetal plate

The data in table (2) also showed the occurrence of wild *Streptopelia senegalensis* in different seasons through one year. The laughing dove was infested with threeacarine species. The main infestation was with *A. streptopelia* in different seasons. The other two mites species were *Ornithocheyletiadubinini*and *Dermoglyphus columbae*. The highest infestations ratio with acarine was obtained in Autumn with ratio (32.5%), ersus in Summer; Spring and Winter they averaged (22.8 ; 16.6 and 4.3%), respectively.Autumn season reveal the highest infestation ratio withacarine and, this could be attributed to the climatic conditions including temperature, relative humidity and rainy weather which were favourable for the survival and development [13].

Table 2: The number and species of infested wild laughing dove in different seasons at Taif Governorate acarine species:

		Extraction methods			
Species of bird	Seasons	Infestation of Acari	.B.B.E*	Tullgren	Number of birds
Streptopelia senegalensis	Winter	*Argas streptopelia-*	1	0	23
	Spring	*Argas streptopelia*	2	0	12
	Summer	*Argas streptopelia - Ornithocheyletiadubinini*	8	3	35
	Autumn	*Argas streptopelia - Ornithocheyletiadubinini-*			
		Dermoglyphus columbae	13	3	40
	Total number				
	of Ectoparasite		24	6	30
Total number of birds					110

In addition, the birds also were infested by different insect species: *Columbicola columbae* and *Goniocotes gallinae* (Philopteridae: Phthiraptera) and *Pseudolynchia canariensis* (Hippoboscidae:Diptera) Fig. (16). The result recorded also that the mite *Hemimyialges* sp. Fig (17). from family Epidermoptidae and order Astigmata which found as a skin parasite for *Pseudolynchia canariensis*. The results of this study wasstrongly agree with the results by Niemelä *et al.* [14] and Soulsby [15].

Extraction Efficiency between Extraction Box of Birds Ectoparasite and Tullgren Extractors, it was observed that Extraction Box of Birds Ectoparasite was higher than the numbers extracted by Tullgren funnels (Table 1 and 2). While Extraction Box of Birds Ectoparasite methods extracted micro-arthropods was more effective. This results may be due to some micro-arthropods were tightly attached to the skin of hosts and the parasitic micro-arthropods never present in feathers of infested wild laughing dove (Fig. 5 and 6). After seven hours, the dead wild laughing dovebirds investigated by Tullgren extraction were moved to Extraction Box of Birds Ectoparasite (E.B.B.E*) extraction for one hour to re-extracted again for macroparasite and microparasite. It was observed that, we got numbers of microparasite specimens were collected.

The number of infested wild laughing dove in different seasons at Taif Governorate with acarine species by using both methods: it was observed that both apparatuses give nearlysimilar proportions and not significantly changes (Table 2). The total incidence ofacarine species in both Extraction Box of Birds Ectoparasite andTullgren funnels was 24 out of 110 and 6 out of 110 respectively.

Concerning The number of infested wild laughing dove in different localities at Taif Governorate with acarine species table (1), revealed that The number of specimens extracted by Tullgren were inefficient for collecting. This result was supported by Barnard [16], While Extraction Box of Birds Ectoparasite methods extracted give more efficient results.

Using Extraction Box of Birds Ectoparasite extraction no parasites escaped due to it was tightly closed, while some author observed fewer swift insects (e.g. ants, spiders, carabid beetles) escaped when loading soil samples into a Tullgren funnel [17].

CONCLUSIONS

Extraction Box of Birds Ectoparasite (E.B.B.E*) extraction is a more efficient device for extracting macroarthropods and microarthropods extraction, both qualitatively and quantitatively. The number of specimens collected by Extraction Box of Birds Ectoparasite extraction is higher than the number collected via Tullgren extractors for all arthropod groups.(EBBE no. 24 out of 110and Tullgren 6 out 110 birds). Tullgren extraction seems to be more suitable for community, diversity and functional studies of soil macroarthropods that require both quantitative Extraction time Soil moisture and Soil temperature and requires a constant power source. We observed fewer swift insects (e.g. ants, spiders, carabid beetles) escaped when loading soil samples into a Tullgren funnel and its extraction time take not less 7 hours.While using Extraction Box of Birds Ectoparasite extraction features are :-

- The application of Extraction Box of Birds Ectoparasite methods extracted either micro-arthropods and macro- arthropods without causing minimal damage compared with the traditional method of previous internationally required killing jumper to complete the separation process and as recommended by the animal welfair organizations [18].

- Providing security and protection for the examiner from direct contact with birds that could be carriers of some Dangerous zoonotic diseases transmitted from birds to humans [19].
- Save time and effort of the researche, so that it was possible to isolate the parasite after hours compared to the previous method which take at leaste 7 hours,[11].
- The innovation can be used on different types of organisms, especially the small size mammals and Can be manufactured in different sizes with the sizes of organisms to be tested.
- Extraction Box of Birds Ectoparasite also can be used as fields Extract using batteries rather than electricity.
- Extraction Box of Birds Ectoparasite, it can be used in research in veterinary screening points to detect any parasites allow epidemics in the country [20].
- No parasites escaped due to it was tightly closed.

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