The Journal of Basic and Applied Zoology

RESEARCH Open Access

Population dynamics of some domestic mites in laboratory culture

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Abstract

Background: Domestic mites are a primary source of allergens indoors all over the world. Extracts of mite allergens are important for diagnosing and treating mites allergy. The effective cultivation processes play a critical role in the final composition of mites' allergen extracts. In order to produce large amounts of antigens of domestic mites, culture techniques of domestic mites were studied. Domestic mites were isolated from collected dust samples and cultured on a fine-ground mixture of dust and sawdust (2:1). While dry yeast, wheat germ, and cornflour were used at a ratio of 2:1:1 for nutrition. Food was placed over a few centimeters of cement base that had been placed on the bottom of the rearing containers. The population growth rates were determined.

Results: Successful methods for mite cultures utilize high protein and carbohydrate foods, an average temperature of (25 ± 2) °C and relative humidity (80 ± 5) %. During a 14-week observation period of mites' culture, the peak in number was obtained after 8 weeks of the culture in *Tyrophagus*, 10 weeks in *Dermatophagoides*, and 12 weeks in *Blomia*; thereafter, the number decreased. Significant differences were found in the growth rates of *Dermatophagoides* ($P = 0.02$) and *Blomia* ($P = 0.03$) in between pure and mixed cultures.

Conclusions: The modifications made to the mixture of diet used for cultivation showed a high yield of domestic mites. Also, the cement layer also is an excellent way to make space for egg laying and prevent mites from escaping.

Keywords: Domestic mites, Mites culture, And population dynamics

Background

Domestic mites include all indoor mites that belong to the subphylum Chelicerata, class Arachnida, subclass Acari, superorder Acariformes, and order Astigmata (Portnoy et al., 2013). The prominent domestic mite species belonging to the *Pyroglyphidae* family include *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), *D. farinae*, and *Euroglyphus maynei*. Other clinically important mites are the storage mites, especially members of the families *Glycyphagidae*, *Acaridae*, and *Echimyopodidae* (Müsken et al., 2002).

Mites are a major source of indoor allergens that sensitize and cause allergic symptoms in people with genetic predisposition worldwide. In order to produce allergen extracts, mites are cultivated and grown in large amounts under precise and controlled conditions in optimal culture media. Proper conditions to grow mites are required to guarantee a suitable allergenic composition of the final product. Mite allergen extracts are essential to diagnose mite allergy and prepare for immunotherapy (Carnés et al,. 2017). Source materials to prepare mite allergen extracts are obtained from inactivated mite cultures and can include different mite components (e.g., fecal pellets, mite bodies, mite parts, egg cases, and skin casts) (Carnés et al., 2008). Dust mites were initially cultured using their natural food source, human skin scales (Spieksma, 1969). Subsequently, alternative culture media were developed, including animal skin scales, dried daphnia, ox liver, fish food flakes, dog food, rodent chow, wheat germ, and fungal cultures usually with yeast (Colloff, 2009) and the

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usage of vitamins or amino acids as a supplement (Chan et al., 2015). However, despite several existing studies on population growth and development of domestic mites, as far as we know, there are no previous studies comparing domestic mite population dynamics in separate and mixed cultures. In the present study, we investigated and determined the growth pattern of the main allergenic mites genera, *Dermatophagoides* (Family *Pyroglyphidae*), *Tyrophagus* (Family: *Acaridae*), and *Blomia* (Family: *Echimyopodidae*) during the period of single- and mixed-population cultivation.

Methods

Isolation of domestic mites

Samples of dust from homes of allergic patients were collected into small plastic bags then sealed and stored at 4 °C till being transferred to the laboratory within 24 h to be examined (El Kersh et al., 2019). Dust samples were sieved to separate the large particles. Mites were isolated from sieved dust samples using Berlese funnels in the Allergy laboratory at the Allergy Medicine Unit, Internal Medicine Department, Ain-Shams University Hospital and the Parasitology Department, Faculty of Medicine, Ain-Shams University. Mites were examined under a stereomicroscope then isolated from the collected dust samples using the tip of a needle for further identification and culture.

Identification of the studied mite species

Dermatophagoides, *Tyrophagus*, and *Blomia* genera were identified according to the keys given by Colloff (2009) as follows: mites have a bilaterally symmetrical, tiny globular body and covered with a translucent cuticle, ranging from 150 to 600 um. Adults have four pairs of legs and fully developed genitalia.

Tyrophagus was identified by having a dorsal transverse groove. With equally sized external and internal vertical setae. *Blomia* has no dorsal transverse groove and has characteristic long dorsal setae. Both the external and internal vertical setae were clustered together on the anterior dorsal margin. That was differentiated from *Dermatophagoides* by the fingerprint pattern of body striations.

Domestic mites' culture

Cultivation of mites in different culture media

We began with four culture replicates per genus, all of them from the same inoculum incubated at 23–25 °C and 75–80% relative humidity in the incubator. Mites were reared in a Petri dish (10 cm × 1.7 cm) with wet paper tissue under its covers; the culture media, according to Arlian et al. (1979), consisted of human hair and active dry granular yeast with a ratio of 1:1. Human hair was washed with soap to remove tonics, cut short, and dried for 24 h before being placed in the culture. Also, Skelton et al. (2010) culture technique was tried using a mixture of fish food and dry yeast granules at 1:1 ratio. Cultures were examined regularly to assess mite growth. Effective rearing culture have been suggested by Stepien and Rodriguez (1973), and from it, we developed the following modification (Fig. 1): Two different sizes of plastic containers were used, a small container 14 cm in diameter (154 cm^2) (rearing container) that was placed inside a large container 17 cm in diameter (227 cm^2); tap water was placed at the bottom of the large container about 1.5–2 cm deep to maintain the required relative humidity (80 ± 5) % in the culture.

We modified the rearing container by making bases consisting of a mixture of commercially available cement and water at a ratio of 2:1, instead of using vermiculite. The mixture was poured into the rearing container then irregular lines were drawn to create cracks before the mixture dries. The diet mixture used was dry yeast, wheat germ, and cornflour at a 2:1:1 ratio. The diet mixture was put on the top of a fine-ground mixture of dust, sawdust in the rearing containers. The isolated mites were placed in the rearing containers. *Dermatophagoides*, *Blomia*, and *Tyrophagus* were reared individually (in separate rearing containers) and mixed culture (in the same rearing containers). The numbers of adult mites from each genus placed in culture were 20 females and 10 males. The rearing containers were left without a lid but covered with the wider container lid. Ventilation holes were made in the lid to maintain the necessary humidity, and the lid was adequately sealed to prevent the escaping of mites. Culture containers were held at an average temperature of (25 ± 2) °C in normal fluctuating room temperature, and in an incubator in the winter. The examination of culture was done in situ using a USB Digital Microscope (× 50–1000).

Mites population growth

Mites of all life stages (larvae, protonymphs, tritonymphs, and adults) were calculated by counting the number of live mites in 50 mg of culture material in each culture container every 2 weeks for 14 weeks using a stereomicroscope (× 10) to assess the growth of the mites and detection of predatory mites' contamination (Fig. 2). The mean number of live mites per sample was calculated from the cultures. Mites were considered living if they were moving. Four replicates have been maintained for each culture.

Statistical analyses

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Statistical presentation and analysis of the present study were conducted, using the mean, standard deviation.

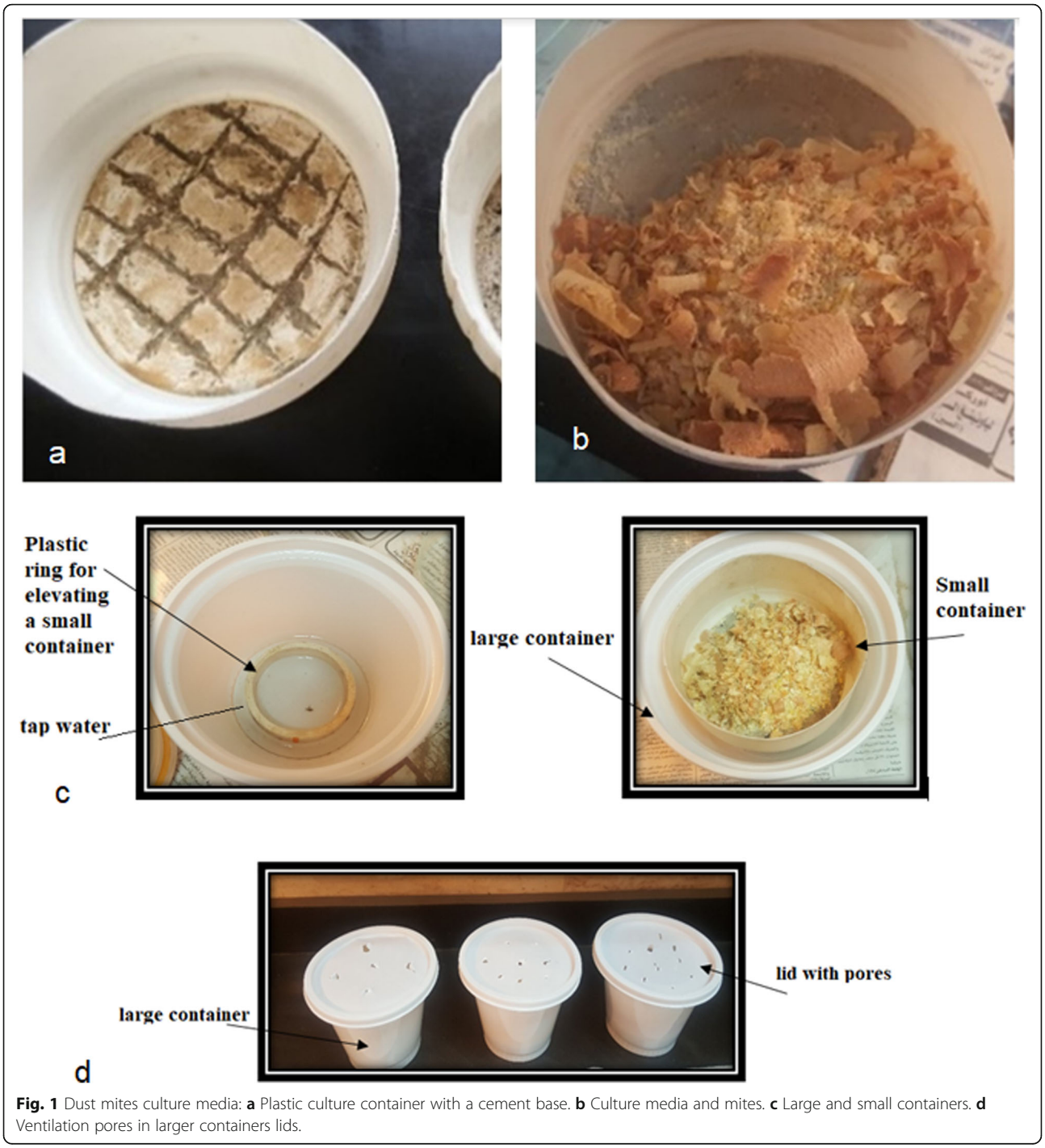

Fig. 1 Dust mites culture media: **a** Plastic culture container with a cement base. **b** Culture media and mites. **c** Large and small containers. **d** Ventilation pores in larger containers lids.

One-way ANOVA was used to detect the difference of growth in the studied three species along the weeks of concerns in the same culture (goodcalculators.com/one-way-anova.calculator).

Paired *t* test was done to calculate the difference of the growth rates in between pure and mixed cultures (https://www.statskingdom.com/160MeanT2pair.html).

Results

Domestic mites' culture

Culture trials performed according to Arlian et al. (1979) and Skelton et al. (2010) showed no growth of mites. The adult mites put in the culture containers either escaped or died when glycerol was used to seal the lid. Successful culture was achieved according to Stepien

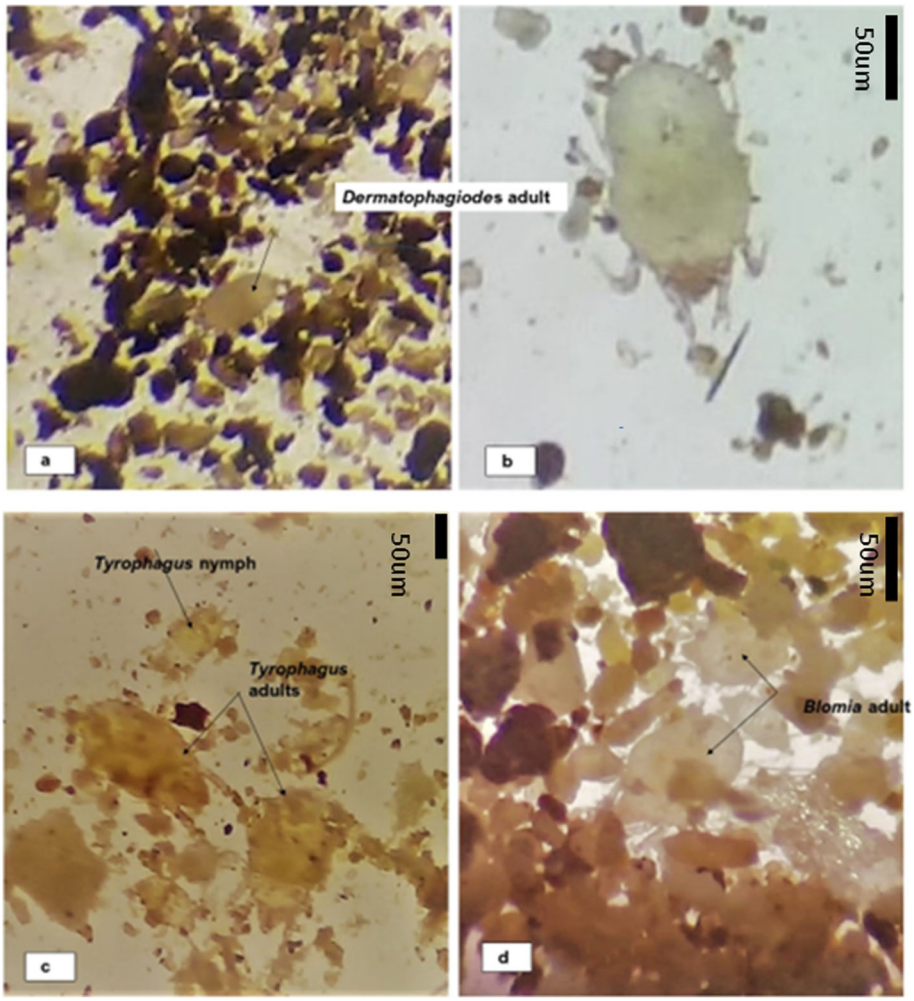

Fig. 2 Domestic mites in culture (**a**, **b**). *Dermatophagoides* mites in culture (× 30 and × 100, respectively). **c** *Tyrophagus* mites (× 30). **d** *Blomia* mites (× 30)

and Rodriguez (1973) with some modifications. Cement was used instead of vermiculite as a base of the rearing container, and irregular cracks were drawn in the cement. The diet mixture used was dry yeast, wheat germ, and cornflour at a ratio of 2:1:1. We have also maintained pure cultures of dust mites, *Dermatophagoides*, as well as storage mites, *Blomia* and *Tyrophagus*. All mites were reared under optimal growth conditions of 25 °C temperature and relative humidity between 75 and 80%.

Population growth in each separate genus culture

The growth process may be divided into three distinct phases: a first latent period takes about 2 weeks, then a second exponential period with a rapid rise in the mite population, and finally a third drop phase. The growth profiles of the studied genera were as follows: *Tyrophagus* population growth rate to the peak was faster than *Dermatophagoides* and *Blomia* where population density reached a maximum in 8 weeks, while 10 weeks for *Dermatophagoides* and 12 weeks for *Blomia* with no statistically significant differences detected in between the three genera along all the studied weeks in their total counts (P = 0.72) despite the difference in peaking counts and timing (Fig. 3 and Table 1).

Population growth in mixed culture

The present study showed that *Tyrophagus* built up a large population within a short time with a peak reaching at 6 weeks but diminished shortly afterward. Following the reduction of the number of *Tyrophagus*, the other two genera of mites started to rise in numbers at the tenth week of cultivation. There was no significant difference in the total counts of the three genera in all the weeks (P = 0.34) though having a different growth curve pattern (Fig. 4 and Table 2).

Statistically significant differences were detected between the growth rates of two genera in pure and mixed

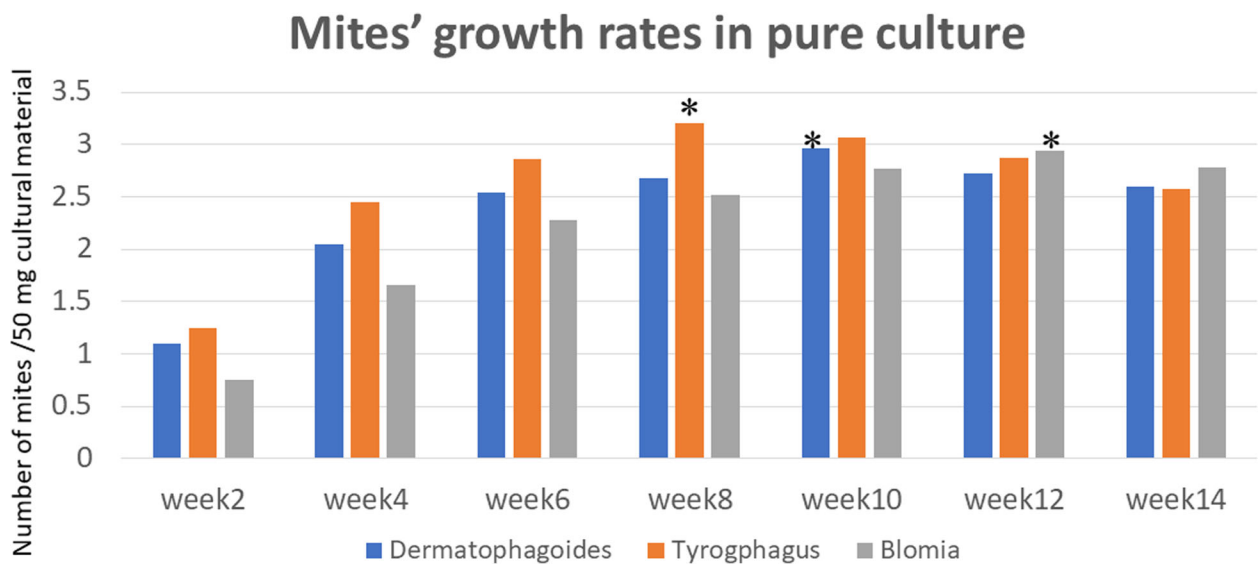

Fig. 3 Growth kinetics of Dermatophagoides, Tyrophagus, and Blomia spp. in 50-mg samples from their pure culture media (25 °C and 75% RH) along 14 weeks. Data represented as the log counts and average of 4 duplicates. *Peak growth

culture types, as $P = 0.02$ in the case of *Dermatophagoides* and $P = 0.03$ regarding *Blomia* cultures (Table 3).

Discussion

Domestic mites are extensively reported as potent allergens worldwide. The living mites, their dead bodies, and their excretory products (glandular secretions and fecal droplets) are considered as sources of active allergens. The occurrence of mites in house dust habitats has been related to several physical and climatic factors, namely temperature and humidity (Dey et al., 2019). It was suggested that the geographical situation of Egypt and its favourable climatic conditions together with other factors may play a major role in the abundance of domestic mite, and consequently, domestic mite allergy occurs more commonly than any other allergen in the Egyptian allergic patients (Kenawy et al., 2012).

In the current study, we found that Arlian et al. (1979) and Skelton et al. (2010) culture techniques did not support the thriving of mite population, due to mites escaping or dying. Successful cultivation was according to Stepien and Rodriguez (1973) with some modification as alteration of the diet components where a mixture of dried yeast, wheat germ, and cornflour with 2:1:1 ratio was used. We also had a problem with mites escaping from their rearing containers, so we placed cement in the base of the containers and created cracks in the cement before dryness to delay mites from escaping and provided areas for quiescent stages of mites and additional place for egg laying.

In the present study, *Dermatophagoides*, *Tyrophagus*, and *Blomia* genera were cultivated. The cultures revealed that the three mites' genera had different population growth rates at 25 °C and 75 ± 5% relative humidity. In each separate culture, the growth curve of the mites showed clearly three phases based on the number of existing live mites: the first being the lag phase, the second, the exponential phase, and the third, the fall phase, which is characterized by a drop in the live mite population due to food limitations as shown by Eraso et al. (1997), Osterberg et al. (2007), and Yella et al. (2011).

The time taken to reach the maximum number of living mites per gram of culture medium was 8 weeks for *Tyrophagus*, 10 weeks for *Dermatophagoides*, and 12 weeks for *Blomia*. While in the mixed culture where the three different mite genera were living in the same rearing container and being allowed to feed on the same

Table 1 Log of cultivated mites counts in pure cultures and statistical analysis

Genus	Week2	Week4	Week6	Week8	Week10	Week12	Week14	F	*P* value
Dermatophagoides	1.09	2.05	2.54	2.68	2.96	2.72	2.59	0.33	0.72
Tyrophagus	1.24	2.45	2.86	3.2	3.07	2.87	2.57		
Blomia	0.75	1.66	2.28	2.51	2.77	2.94	2.78		

One-way ANOVA to detect the difference of growth in the three species along the studied weeks in the pure culture (P value ≥ 0.05 non-significant)

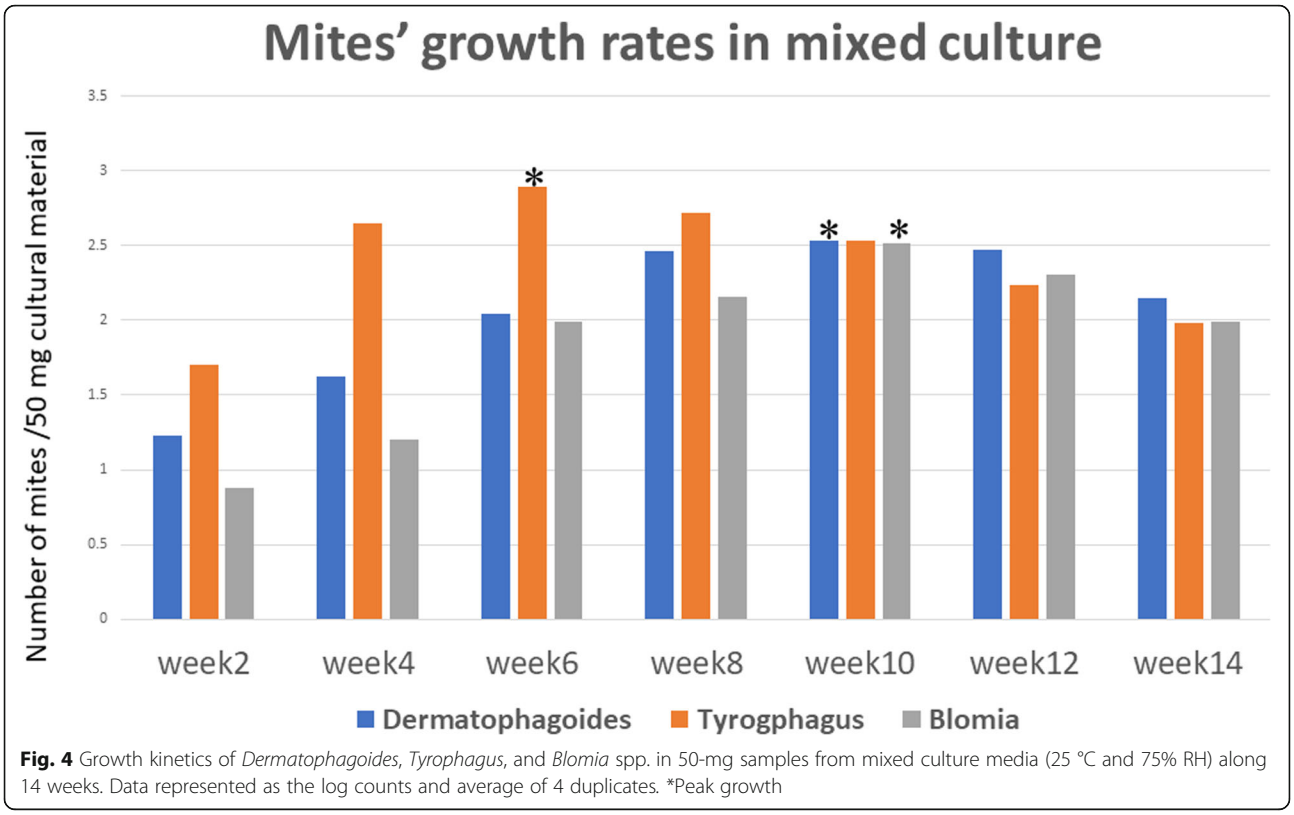

Fig. 4 Growth kinetics of *Dermatophagoides*, *Tyrophagus*, and *Blomia* spp. in 50-mg samples from mixed culture media (25 °C and 75% RH) along 14 weeks. Data represented as the log counts and average of 4 duplicates. *Peak growth

ingredients, two genera showed a significant difference in their counts between pure and mixed cultures so they could be considered as competitors for each other. This study found that *Tyrophagus* built up a large population within a short time but diminished shortly afterward. Following the reduction of the number of *Tyrophagus*, *Dermatophagoides* and *Blomia* started to increase in numbers.

As selection of the culture media is one of the important factors for successful mites' culture. The result of the present study showed that the mixture of a finely-ground mixture of dust and sawdust (2:1) and a diet mixture composed of dried yeast, wheat germ, and cornflour with 2:1:1 ratio gave a high yield of cultivated mites. The mixture of yeast powder and washed human hair did not support growth and multiplication of mites. This may be due to some human hair components preventing the mites' development.

A study Eraso et al. (1997) found that at 23–25 °C and 75–80% RH *Dermatophagoides* cultures reached maximum density at 16–18 weeks (grown on rat and mouse fodder and dried yeast powder). However, this is considerably longer than it took our cultures to mature. Ree and Lee (1997) found that the maximum yield of *Tyrophagus* was obtained in the mouse food powder showing an increase in number after 10 weeks, and the recommendable time of harvest is in weeks 9–10. Ree et al. (1997) showed that the mixture of fish food powder and dried yeast with 1:1 ratio gave the highest yield of *Dermatophagoides*.

Similar to our results, Osterberg et al. (2007) found that the maximum yield of *Dermatophagoides* during culturing occurred between 40 and 60 days (5.7–8.6 weeks) of cultivation at 75% RH and 25 °C, and then the yield declined over the following 40 days. Petrova-Nikitina et al. (2011) showed that the maximal

Table 2 Log of cultivated mites counts in mixed cultures and statistical analysis

Genus	Week2	Week4	Week6	Week8	Week10	Week12	Week14	F	*P* value
Dermatophagoides	1.23	1.62	2.04	2.46	2.53	2.47	2.15	1.16	0.34
Tyrophagus	1.7	2.65	2.89	2.72	2.53	2.24	1.98		
Blomia	0.88	1.2	1.99	2.16	2.52	2.31	1.99		

One-way ANOVA to detect the difference of growth in the three species along the studied weeks in the mixed culture (*P* value ≥ 0.05 non-significant)

Table 3 The difference between growth rates in pure and mixed cultures for each studied mite

		Dermatophagoides	*Tyrophagus*	*Blomia*
Mites counts ± SD	Pure culture	2.1662 ± 0.8317	2.3888 ± 0.8701	2.0363 ± 0.9297
	Mixed culture	1.91 ± 0.6435	2.2138 ± 0.632	1.7188 ± 0.6912
Statistical analysis	T	2.9415	1.1499	2.7959
	P value	0.02*	0.2879	0.03*

Paired *t* test to calculate the difference of the growth rates in between pure and mixed cultures regarding each mite genus (*P value ≤ 0.05 significant)

population density was at 14 weeks of *Dermatophagoides* cultivation. However, other factors also could influence mite fluctuations, such as using a different culture media and different size of rearing containers.

Selection of the rearing container size is also an important factor for successful culture of domestic mites. The size of rearing container determines the surface area of the media when the same quantity of media is used, as the results of the present study showed that a 14-cm-diameter (154 cm^2) plastic container gave the highest yield of cultivated mites. Meanwhile, culture in a clean dried small Petri dish (10cm × 1.7 cm) showed failure of growth and mites escaped. Ree and Lee (1997) showed the important of using the appropriate size of rearing container where the container with a larger surface area (14 cm in diameter, 154 cm^2) gave 3.9–5.1 times higher yield in *Dermatophagoides* compared to that with a smaller surface area (10 cm in diameter, 79 cm^2).

Conclusions

We found that mite genera which were reared individually showed the best growth pattern than those reared together in the same container. *Tyrophagus* showed faster growth rates in culture compared to other reared species. Also, our culture trials showed that mite-culturing diet directly affects population growth. Therefore, culture conditions and growth media are significant factors when designing species-specific house dust mites' cultivation methods for the production of structured mite extracts for commercial and research purposes.

Acknowledgements

The authors would like to thank all the laboratory staff of the Allergy laboratory at the Allergy Medicine Unit, Internal Medicine Department, Ain-Shams University Hospital and the Parasitology Department, Faculty of Medicine, Ain-Shams University for all their assistance and support.

Authors' contributions

AA was responsible for the sample collection, culture maintenance, and data acquisition. EZ supervised the design of the study and data interpretation. Ak helped in the laboratory work, data acquisition, and drafting of the manuscript. NE structured the concept and study design, interpreted the whole data, and made the statistical result and the final manuscript revision. The authors read and approved the final manuscript.

Funding

The research work was self-funded.

Declarations

Ethics approval and consent to participate

The study followed the regulations of the Egyptian Ministry of Higher Education and obtained the required approval of the ethical committee of the Faculty of Medicine Ain-Shams University. The reference number is not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 1 May 2020 Accepted: 17 February 2021
Published online: 02 March 2021

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