A NEW TECHNIQUE FOR ISOLATION OF MITES EXPLOITING THE DIFFERENCE IN DENSITY BETWEEN ETHANOL AND SATURATED NaCl : QUALITATIVE AND QUANTITATIVE STUDIES

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TECHNIQUE FOR ISOLATING MITES

ABSTRACT : Qualitative and quantitative experiments using a new flotation method for isolating mites from Berlese extracts, museum storage jars, house-dust and laboratory cultures are described. The results show the extractive efficiency to be 97-98 %. The advantages of this technique over other known techniques are dicussed.

TECHNIQUE D'ISOLEMENT DES ACARIENS

RÉSUMÉ : Des expériences qualitatives et quantitatives utilisant une nouvelle méthode d'extraction des acariens à partir d'extraits de Berlese, de solutions alcooliques ayant contenu divers animaux, de poussières de maison et de cultures de laboratoires sont décrites. Les résultats montrent une efficacité d'extraction de 97-98 %. Les avantages de cette méthode sur les autres méthodes connues sont discutés.

INTRODUCTION

FAIN and HART (1986) described in a recent preliminary note a new method for extraction of mites and other microarthropods from various media such as Berlese extracts, house-dust and laboratory mite cultures. This new method is developed from the method described previously by FAIN (1966) which has been improved by soaking samples in 80 % ethanol (ethyl alcohol) for several hours before the addition of saturated salt solution, thus exploiting the difference in buoyant density between 80 % ethanol and saturated NaCl to float the mites or microarthropods under investigation. We describe herein experiments which qualitatively and quantitatively characterize the technique.

MATERIALS AND METHODS

For ethanol samples (Berlese extracts, Museum storage jars etc.) the supernatant can simply be decanted and replaced directly with saturated Nacl, however for dry samples (house-dust, laboratory mite cultures etc.) it is necessary to soak samples in 80 % ethanol for at least 4 h before the addition of the salt solution. Details of our experiments to test the efficiency of this technique are as follows :

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1. *Ethanol Samples from Berlese Extracts and Museum Storage Jars*

The total ethanol sediment from Berlese extracts or museum jars may be used, however in these experiments a total mite count was not necessary and therefore smaller samples were used. 2 ml samples were pipetted into glass cylindrical tubes of 12 cm × 3 cm. 80 ml saturated NaCl solution was then added to each tube, and the tubes were left for 10 min to allow the mites to float to the surface. After this time each sample was decanted equally into four small petri dishes of 60 mm × 15 mm, taking care to turn the tubes while doing so in order to remove any mites on the sides. The four petri dishes for each sample were than examined under a dissecting microscope. All mites floating in the dishes were counted, and the sediment in the fourth dish was also examined for mites which had not been successfully floated. These experiments were repeated three times.

Mites started to sink after 1/2 h, but significant numbers did not sink until 2 h after the addition of saturated NaCl, leaving ample time for examination of the samples which took 15-30 min, depending on the number of mites and quantity of sediment present.

2. *Dry House-Dust and Laboratory Mite Culture Samples*

Tests showed that dry samples of 0,1 g house-dust or mite culture samples gave the best results (HART, unpublished results). Larger samples are less efficient, probably due to mites being caught in dust or food particles (BRONSWIJK *et al.*, 1978).

0,1 g samples of mite-free house-dust or of material used for rearing laboratory mite cultures (a 1 : 1 : 1 mixture of Tetramin, flour and fresh yeast) were added to glass cylindrical tubes of 12 cm × 3 cm. 80 ml of 80 % ethanol was added to each tube into which were incorporated 50 live mites. 25 *Dermatophagoides pteronyssinus* (♂ and ♀) and 25 *Glycyphagus domesticus* (♂ and ♀) mites were used to test the flotation of different sized mites. The samples were then mixed well and left for 24 hr to allow the ethanol to impregnate the bodies of the mites. Four hours was the minimum time necessary for ethanol impregnation, however a 24 h impregnation was most convenient for our purposes.

After 24 h, the ethanol was carefully decanted, with minimum disturbance of the sediment, and examined under a dissecting microscope for mites. 80 ml saturated NaCl was then added to each tube. After 10 min each sample was decanted into four small petri dishes and examined for mites as described in section 1.

This method (without addition of live mites) was repeated using both 0.1 g house-dust known to contain mites and 0.1 g laboratory mite cultures, in order to test the efficiency of this method when live and dead mites an addition to exuviae and eggs are present. All experiments were repeated three times. The mite separating funnel used by FAIN (1966) may also be incorporated with this method if desired.

RESULTS

The technique was suitable for large and small mites since *D. pteronyssinus* and *G. domesticus* floated equally well. Consequently, all calculations were made on the total numbers of mites found.

As seen in Table 1, the technique was extremely efficient in the flotation of mites, with an average of 97-98 % mites floating, and only 2-3 % found in the sediment. In no case were mites found in the ethanol supernatant. Furthemore, mites were easily counted since there was very little flotation of house-dust or of organic or vegetable matter from the Berlese extracts, museum jars or mites cultures. In samples B, E and F, in addition to live mites, dead mites, exuviae and eggs also floated, and these were included in the calculations.

DISCUSSION

In the method described previously (FAIN, 1966) we used an aqueous saturated solution of NaCl.

TABLE 1 : Percentage of total mites found in saturated aqueous NaCl supernatant and sediment.
In samples B, E and F, live mites, dead mites, exuviae and eggs are included in the figures.
Details of the Berlese samples are as follows :
1. Nest of *Cecropis semirufa neumanni*, 1967
2. Nest of *Talpa europa*, 1981
3. Nest of *Cinnysis venustus falkenstaini*, 1967
Details of the museum storage jars are as follows :
1. *Castor fiber*, 1933
2. *Taupe*, 1968
3. *Molothruis badiue*, 1965

Test		Total Mites	Mites	
			Supernatant	Sediment
A. Berlese	1.	454	94.71	5.29
	2.	76	96.05	3.95
	3.	101	100	0
B. Museum Jars	1.	264	98.10	1.89
	2.	42	100	0
	3.	21	100	0
C. Mite-free culture + 50 mites	1.	50	100	0
	2.	50	90	10
	3.	50	100	0
D. Mite-free house-dust + 50 mites	1.	50	100	0
	2.	50	90	10
	3.	50	94	6
E. Mite culture	1.	563	98.28	1.72
	2.	388	95.57	4.43
	3.	432	98.59	1.41
F. House-dust	1.	215	97.99	2.01
	2.	197	100	0
	3.	199	99.67	0.33
Mean ± standard deviation			97.45 ± 3.30	2.61 ± 3.33

The density of this solution is 1.2, which is nearly identical to that of mites (DE GRIDELET-SAINT GEORGES, 1976). This very slight difference in density probably explains the relatively low extractive efficiency of this method. Another reason of the low efficiency of this method lies in the fact that mites are strongly attached to dust particles which prevents them from floating to the surface of the salt solution (BRONSWIJK *et al.*, 1978). Our observations show that the use of small, carefully mixed samples, facilitate the detachment of mites from house-dust. FAIN's technique using saturated NaCl has also been greatly improved by initially soaking dust samples in 80 % ethanol before suspending the mites in the salt solution. Presumably, by impregnating the mites with 80 % ethanol, their density is lowered to that of the ethanol, i.e. 0.86. This is also the case in samples of Berlese extracts and museum jars where mites are stored in ethanol. By then adding saturated NaCl with a density of 1.2 we obtain the equivalent of a solution of density 1.34 which is sufficient to allow a high extractive efficiency of mites.

Due to the importance of mites in allergies, many other techniques have been described for their extraction from house-dust. The density of the various liquids used in established flotation analyses of house-dust is usually 1.2-1.3 (reviewed BRONSWIJK *et al.*, 1978). One method (OSHIMA, 1970) uses carbontetrachloride (CCl_4) to obtain a density of

1.3-1.6. The fumes of CCl_4 are however toxic, which deters many workers from employing this technique. Estimates of the extractive efficiencies of these techniques range from 60-90 % mites recovered (WHARTON, 1976), however these techniques can be rather complicated, requiring laborious multiple sieving, centrifugation, flotation or filtration steps. The great advantages of the new technique described herein are : (i) a density of 1.34 ; (ii) a recovery rate of 97-98 % mites ; (iii) simplicity ; (iv) speed and (v) safety. In addition, the efficiency of this technique in floating not only live mites, but also dead mites, exuviae and eggs, renders it applicable to ecology studies of mite populations.

Several methods have also been developed for the collection of mites from culture media (reviewed ARLIAN *et al.*, 1979). This is particularly important in the case of *D. pteronyssinus*, for obtaining mite extracts for use in clinical studies of house-dust allergy. The main problem here is to obtain mites uncontaminated by culture media and in large quantities. Using the technique described herein, dust or other organic or vegetable particles did not float to the saturated NaCl surface, resulting in a very clean surface on which mites could be easily seen and collected. There exists, therefore, the possibility that further development of this technique could enable its use in obtaining mite extracts for clinical studies.

Ethanol from Berlese extracts and museum jars is normally examined directly under a dissecting microscope for mites. This can prove to be difficult if the sediment contains a large quantity of debris. By simply decanting the ethanol and adding saturated NaCl, mites in such samples can be easily observed for counting and collection. This simple enhancing step has not been reported previously.

In conclusion, many techniques have been described for extracting mites from various media. All have advantages and disadvantages depending on e.g. equipment and time available, whether a quantitative or qualitative analysis is required. Few, however, are as straightforward and effective as that described herein, but most could probably be enhanced, simply by lowering the mite density by 0.34 with a preliminary soaking of samples in 80 % ethanol.

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