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THE SIMPLEST «FIELD» METHODS FOR EXTRACTION OF NEMATODES FROM PLANTS, WOOD, INSECTS AND SOIL, WITH ADDITIONAL DESCRIPTION HOW TO KEEP EXTRACTED NEMATODES ALIVE FOR A LONG TIME

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The simplest modification of the dynamic extraction method using cottonwool filter based on the Baermann funnel principle, is described. This modification excludes the funnel because a great share of sticky worms attach to sloping walls of a funnel and thus do not reach the collector Eppendorf tube. But the main principle of the Baermann funnel is used, i. e. sinking down of actively moving heavy narrow bodies via wide holes of filter and thus separating the active worms from passive non-Brownian moving substrate particles, which do not pass the filter and remain above it. This principle is illustrated because it has never been described before. In the proposed modification any sloping walls in the extraction paths are excluded and thus the probability to attach sticky nematodes to walls is also excluded; only cylindrical equipment with abrupt vertical walls is used; procedures are extremely simplified to be user-friendly for beginners: only filter (cotton pads), Eppendorf tubes, plastic glasses and narrow PVC tubing are applied. The new simplified modification allows one to collect nematodes by non-professional workers, e. g. in Polar expeditions without microscopic study of results. As an addition, an efficient method to maintain extracted nematodes alive is proposed, using the «effect of water film» in foam rubber inside the Eppendorf tube. To maintain nematodes alive during several months it is recommended to suppress bacteria via addition of 0.2—0.4% formaldehyde solution and then keep the tube with nematodes in a refrigerator.

Key words: nematodes, field extraction technique, nematode collection in Polar expedition, Baermann funnel modification, alive nematode storage.

САМАЯ ПРОСТАЯ МЕТОДИКА ПОЛЕВОЙ ЭКСТРАКЦИИ НЕМАТОД ИЗ РАСТЕНИЙ, НАСЕКОМЫХ И ПОЧВЫ, А ТАКЖЕ МЕТОДИКА ДЛИТЕЛЬНОГО СОХРАНЕНИЯ ЭКСТРАГИРОВАННЫХ НЕМАТОД ЖИВЫМИ

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Предложена методика экстракции нематод, представляющая модификацию вороночного метода Берманна. Модификация исключает воронку, но сохраняет волокнистый фильтр как основу разделения нематод и посторонних частиц за счет активного проникновения нематод сквозь поры фильтра с одновременным оседанием под действием силы тяжести. Иллюстрирован ранее не объяснимый принцип действия старого эмпирического метода Берманна, и в новой модификации произведено упрощение методики с одновременным повышением эффективности: выход нематод гораздо выше, чем в классическом методе воронки, так как исключает соприкосновение липкого тела червя с наклонной поверхностью и строго следует принципу оседания нематод в цилиндре с вертикальными стенками. Новая модификация методики допускает «слепое» использование без просмотра результата на месте и может быть использована не профессионалами, например, техническим персоналом дальних полярных экспедиций. Дополнительно предложен эффективный метод сохранения экстрагированных нематод живыми, созданием в пробирке хранения «эффекта водной пленки». Для сохранения нематод живыми в течение нескольких месяцев необходимо добавить водный раствор формалина в концентрации 0.2—0.4 % формальдегида для подавления развития бактерий.

Ключевые слова: нематоды, методы полевой экстракции, сбор нематод в полярной экспедиции, воронка Берманна, сохранение живых нематод.

Methods of extraction of nematodes, i. e. separation of roundworms from the substratum, may be divided in three groups: centrifugal, dynamic and flotation ones (Van Bezooijen, 2006; OEPP/EPPO 2013; Ryss, 2015; De Ley et al, 2016). Centrifugal methods are based on the mass density (specific gravity) of the extraction solution, which may be equal to the specific gravity of nematodes (s. g. 1.13—1.20 g/sm^3) or more than this value. Centrifugal flotation includes two phases: at the first phase after centrifugation in water nematodes and substratum are sedimented to separate them from more floaty organic matter, and at the second stage a sediment of the first stage are mixed with saturated $MgSO_4$ solution (s.g. 1.15—1.2 g/cm^3) and then centrifuged; as a result nematodes float up and the supernatant is to be quickly washed with water on a 20 μm sieve, to avoid nematode bodies distortion from concentrated saline solution. The method needs a centrifuge and thus can be used only in the laboratory; as a solution of the heavy specific gravity (s. g.) the saturated solution of magnesium sulfate (MgSO4 * 7H20) or the sucrose solution are used commonly. The flotation method includes the flux of air bubbles through the water suspension of nematodes and the substratum; nematodes (as inhabitants of water film) are attached to bubbles and float up with bubbles to the water surface; then the upper layer of the suspension is poured and it is washed again on a sieve to concentrate the ne-

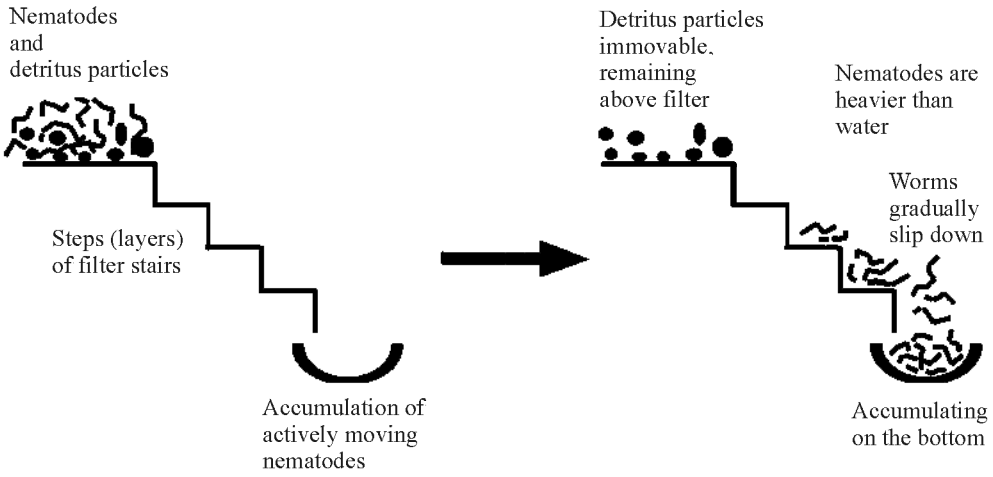

Fig. 1. General principle of Bearmann Funnel technique. Active moving nematodes sink «step by step» through porous filter (here illustrated as stair steps), whereas motionless mineral and organic ground particles remain above the filter (on the upper stair, left).

matode suspension. This method needs large consumption of water saturated with air under pressure and again can be used only in laboratory conditions.

The oldest and the most economic among the extraction techniques is the dynamic method that is known commonly as «Baermann funnel». While using this technique, nematodes pass through a fibrous (cellulose or cotton) filter sinking down in a container filled with water whereas the particles of substratum both light and heavy do not pass and they are located above the filter. When I started to work in nematology I asked my teacher E.Kirjanova and E. Krall why only nematodes can pass the filter and not the soil particles, because Baermann as the author of technique did not give an explanation of his method principle. Later when I did my research in foreign nematology laboratories and was participating in a nematological meeting I addressed the same question to leading nematologists. Nobody could explain the key principle of Baermann's technique, however, the specialists shared an opinion that the funnel method is purely empirical, classical, and it allows to extract only part of nematodes because the latter have ability to penetrate small pores in the substratum.

When I looked at nematodes locating on the sieve I saw how nematodes permanently flexing like snakes, occasionally insert the anterior body part via the sieve mesh. Gradually their anterior ends and other parts follow the anterior one during undulation movement and under gravitation. Thus all the nematode bodies pass into the lower plane of sieve meshes occurring below a sieve and then sink down to the bottom because their specific gravity is higher than that of water (1.15 g/cm^3 *vs* 1.0 /cm^3). The particles of the substratum are not movable and they are located on the upper surface of the filter (fig. 1). I came to a conclusion that while the filter itself is an analogue of sieve but it is more irregular in its mesh shape and size than the nylon one. Thus in Baermann extraction technique the same process of sinking down of actively flexing thin body with a diameter smaller than a sieve mesh, may take place, like a ball is rolling down the stairs, while the fixed dust remains upstairs (fig. 1; Ryss, 1986, 2015). Accepting such assumption, I tried to simplify the Baermann method to make it usable by

non-professional collectors in a field survey. Simultaneously some simple ways were used to improve the method's efficiency.

The goal of this paper is a description of a simplified method of field extraction using the above-mentioned principle of «Baermann funnel».

The main inconvenience of the Baermann technique was exactly a funnel itself, which plays a role of a concentrator of the nematode suspension. Nematodes pass the filter and then sink down onto a sloping inner surface of a funnel and then they roll down into a lower container. But because of their sticky surface coat they attach to the inner funnel surface (especially to a plastic one) and thus they do not sink with 100 % efficiency. This effect was not recognizable in the 19th century when the method was proposed, because at that time funnels were prepared from glass, while plastic materials were invented later. However, now in field conditions the plastic funnels are used because they are lightweight and shatterproof. Nematodes cling immediately to a plastic surface and after that they cannot be moved by a needle without rupturing the nematode body. The surface coat acts as a glue attaching the nematode to the plastic. This fact is well known to all nematologists working with plastic equipment: Petri dishes and slides.

Because of this in the modification of Baerman technique proposed below the same cottonwool filter is used as in the original technique, but all walls of the extraction chambers are sharply vertical, to exclude the sedimentation of nematodes on any surfaces except of the bottom. While the filtration of nematodes through cottonwool needs the dispersion of substratum over the maximum area of the filter, whereas the final suspension has to be concentrated in a narrow Eppendorf tube, so the extraction procedure is logically divided into two subsequent phases: a) extraction on a wide filter area, b) concentration of the purified nematode suspension via the sedimentation in a narrow cylindrical tube with the Eppendorf tube attached from below as a tip. From a previous version of the method (Ryss, 2015) the newly proposed technique differs in the use of a double filter made from the standard cotton pads from supermarket, as well as in cheaper and more reliable materials of extraction chambers and tubes. Application of this wares saves time in field monitoring and make the extraction easier and cheaper.

1. METHOD OF NEMATODE EXTRACTION

a) Extraction of nematodes on a filter in the «sieve» chamber

To prepare the extraction chamber four 250 ml transparent plastic cups (for water and juice) are used. Cups are sold in a package inserting one into another because of their slightly subconical shape, very close to the cylinder. Three cups are rotated bottom up and then their bottoms are removed with a utility knife closely to the border of the bottom circle; however, the fourth (lower) cup remains uncut (fig. 2, *A*, *B*). Two cut cups are dipped in water with cut ends to insert the filter more easily. Cotton pads (which are sold in supermarkets in column packages) are used as filters. Each pad is double. It contains two tissue layers with a loose cottonwool layer in-between. To prepare a filter, a pad is divided in two layers and one layer is inserted on the previously moistened cut bottom of a cup; borders of this pad layer have to be extended equally beyond the edge of the cut bottom. These borders are pressed with hand and thus fixed on the mois-

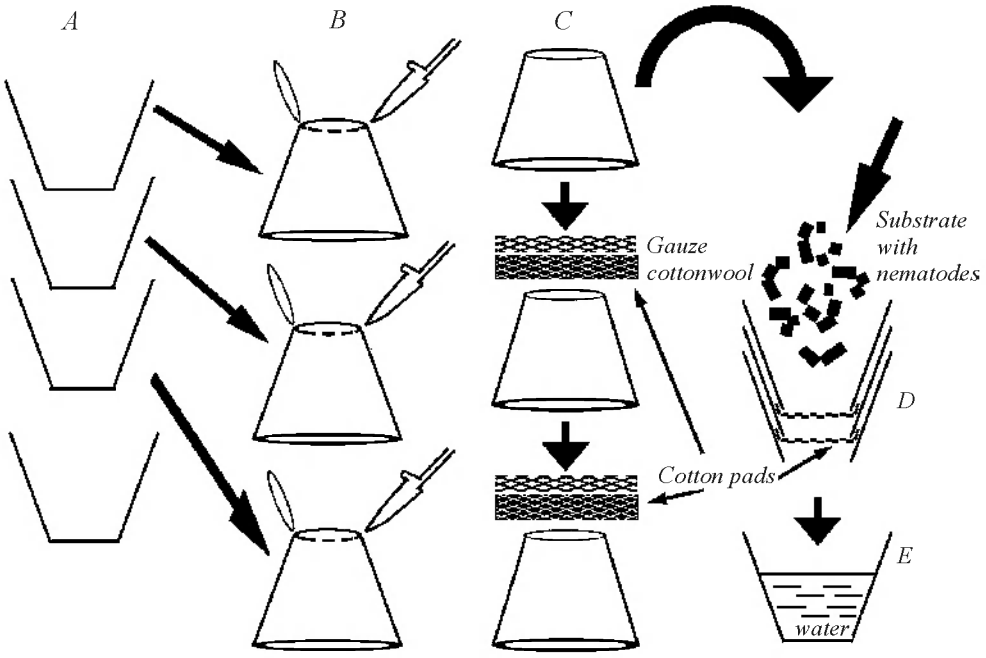

Fig. 2. Nematode extraction.

A — four 200 ml subconical plastic beakers which may be inserted one into another. *B* — bottoms of three beakers are to be cut with a scalpel; the fourth one will be a water container. *C* — three clipped beakers are turned bottom up and two cotton pads are put on, thus pads are jammed between the beakers, forming an extraction «sieve». *D* — the substrate with nematodes is put into the «sieve» (which turned again bottom down), and the «sieve» is immersed into the fourth beaker filled with water, for 12—24 hours. *E* — removing of the «sieve», active nematodes passed through a filter and retain in water in the beaker below.

tened sides of the cup. The second cut cup is inserted atop and thus the first pad layer is jammed between two cut cups, forming a «sieve» (fig. 2, *C*). While the upper cup bottom is also moistened, the second pad layer is inserted above, and the third cut cup is placed atop (fig. 2, *C*). As a result, the «sieve» chamber of extraction is ready. It must be rotated in the normal position (bottom down), the substratum with nematodes (soil, wood chips, or a cut insect) is to be placed in the chamber, and the latter is placed into the fourth cup filled with water (fig. 2, *D*, *E*). The chamber sink into water of the fourth cup under its own weight, no pressing must be used to avoid filter damage or forming air bubbles under the filter. The double filter is used to avoid occasional mistakes arising in preparation of the chamber and the substratum insertion into it, e. g. filter damage or filter border release from jaw between cups. Time of extraction must be not extremely long otherwise some nematodes may die because of the oxygen deficiency in the water column (the majority of nematodes live usually in the water film of air bubbles and not in water itself); to obtain clean water suspension of live nematodes time of extraction has to be 6—12 hr. But if the goal of extraction is to evaluate the total nematode number in substratum, and if the deaths of extracting nematodes are acceptable, the extraction of 24 or 48 hr can be used. To extract nematodes from insects it is better to replace water by the physiological saline solution isotonic to hemocoel (0.9 % NaCl) and to use only 3—5 hr extraction to avoid a substratum decay.

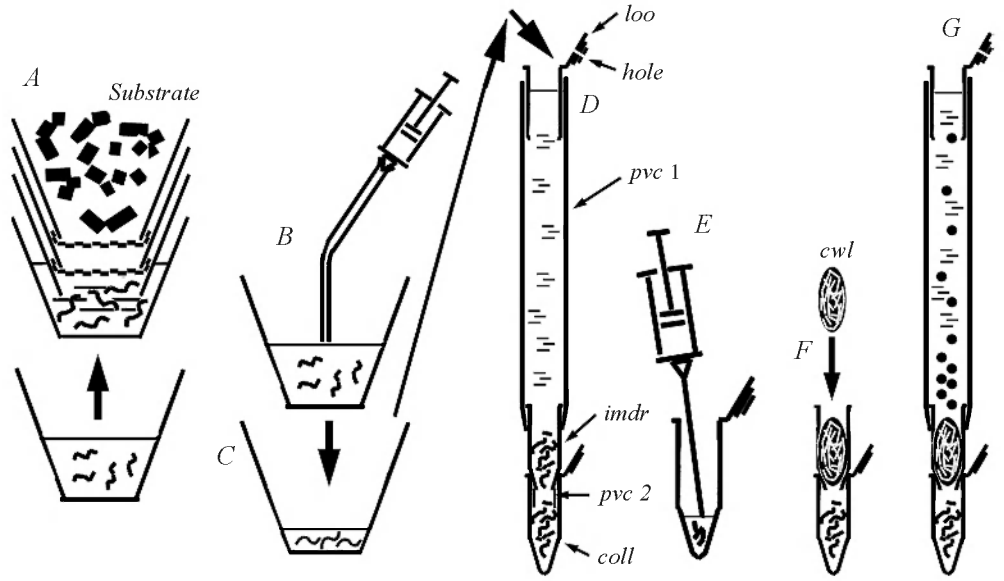

Fig. 3. Final phases of extraction.

A — quick removal of the «sieve»; nematode suspension retain in bottom beaker; *B* — using a syringe with capillary tubing, a slow removal the upper layer retaining 1 cm water layer (*C*); *D* — the suspension shaking and pouring it into the 25 cm long PVC tubing construction with 1.5 ml Eppendorf tube at the end, for 1 hr; *E* — a slowly removal the upper layer of water with a syringe from the Eppendorf tube until its conical part, thus creating an air volume for the nematode respiration; *F*, *G* — additional cleaning the dirty suspensions: *F* — insertion of the cottonwool wad into the cut intermediator Eppendorf tube, previously filled with water. *G* — a 25 cm PVC tubing inserted on the double Eppendorf tube column with the cottonwool wad inside, the dirty nematode suspension is poured in it, maintaining the vertical position of a tubing construction for the 5 hr extraction. Signs marked parts of the extraction tube construction: *coll* — 1.5 ml Eppendorf tube-collector; *cwl* — cottonwool tampon; *hole* — a hole in upper «loop» Eppendorf tube; *imdr* — intermediator Eppendorf tube (cut at both ends); *loo* — «loop» Eppendorf tube; *pvc 1* — main 25 cm PVC tubing (diam. 10 mm); *pvc 2* — 1 cm PVC tubing (diam. 8 mm).

b) Concentration of the extracted suspension in a cylindrical flexible plastic tubing ending with an Eppendorf tube

After extraction all nematodes passing through the wide filter are inside of the chamber. They must be collected into a narrow 1.5 ml Eppendorf tube. To concentrate the nematode suspension, a 20—25 cm PVC cylindrical tube is used with a diameter equal to the outer diameter of the Eppendorf tube, i. e. 10 mm. Nematodes sink down in the column of water in a tube under the force of specific gravity exceeding this value for water (1.13—1.25 g/cm^3 vs. 1.0 g/cm^3) (fig. 3, *D*). While floating down in a tube the suspension can be additionally cleaned from debris particles occasionally passing the filter slits. Slits may appear as a result of filter rupture during chamber preparation, or during the immersion of substratum into the chamber, or as a result of activity of other invertebrates: mites, insect larvae, oligochaetes. An additional filter is inserted into the tube only as an exception if the pollution of the suspension is evident; usually the suspension is clean and the simple sedimentation without any additional filter is to be used. Therefore here below I describe both versions of the tube application: a) with simple sedimentation in a long tube, b) with additional cleaning of the suspension using a cottonwool filter inserted in a tube just before the collector container (i. e. Eppendorf tube).

2. THE TUBING SYSTEM CONSTRUCTION

The tubing system parts are collected from an electrically insulating PVC tubing, which is an extraordinary cheap material that can be bought in electric supermarkets. For extraction tube construction the PVC tubing of two different diameters are used, taking into consideration that the final place of the nematode suspension is a 1.5 ml Eppendorf tube: the main 25 cm PVC tubing part has its inner diameter matching the outer diameter of the Eppendorf tube (10 mm) while the 1 cm PVC intermediate tubing part joining the main construction with the Eppendorf tube must have an outer diameter that is equal to the inner diameter of the Eppendorf tube (8 mm). The main part of the construction is a 25 cm PVC tubing of 10 mm diameter (fig. 3, *D*). From below it is necessary to attach the 1.5 ml Eppendorf tube aimed to collect the nematodes (tube-collector), and atop another Eppendorf tube with cut conical part is to be inserted (fig. 3, *D*). The upper tube is used as a loop to hang the construction on a hook, for what it is necessary to pierce a hole in a tube cap for a hook. It is important to foresee the section with an additional cootonwool filter before the tube-collector. To arrange such section, an additional cut 1.5 ml Eppendorf tube is used, which is an intermediator tube between the main 25 cm PVC tubing part (diam. 10 mm) and the Eppendorf tube collector allowing to remove easily the latter from the main PVC tube after all nematodes will be collected down. The intermediator Eppendorf tube has to be cut both atop removing the cap and the upper rand, and from below removing two thirds of the conical part. The upper end of the intermediator tube is inserted into the main 25 cm PVC tubing from below, and 1 cm of the PVC tubing of 8 mm diameter is put on the cut tip of the intermediator Eppendorf tube. While this narrow 8 mm PVC tubing exactly matching the inner diameter of the Eppendorf tube-collector, the latter may be easily put on the construction from below (fig. 3, *D*, *G*). It is necessary to attach the sticky label to the collector tube cap containing the information on the sample or its database code. After pouring of the nematode suspension from the chamber into the composite tubing construction, the latter is to be hanged in a vertical position on a hook passing through a hole in the upper «loop» Eppendorf tube.

3. CONCENTRATION OF NEMATODES IN THE EPPENDORF TUBE VIA A SIMPLE SEDIMENTATION IN TUBING SYSTEM

After the exposure of substratum in the extraction «sieve» chamber (overnight or more), the chamber must be quickly and abruptly removed from the lower cup with water (fig. 3, *A*). To that moment nematodes in mass have already passed through the filter and settled on the bottom of the cup. The chamber has to be disassembled, the sample substratum and the used filter must be thrown away, but the cut cups may be kept for future extractions after washing and drying. The water suspension of nematodes in the cup has to stay for 20 min to allow nematodes settle down after the chamber removal. Then the upper layer of water must be carefully removed until 1 cm depth using a syringe with a flexible tubing replacing the standard needle (fig. 3, *B*). The flexible tube (from a disposable blood transfusion system set, 3 mm in diameter) can be bought to-

gether with a disposable syringe at a pharmacy. The tube is to be cut into 10—15 cm segments used to suck out water. The nematode suspension on the bottom of the cup needs a secondary sedimentation to collect nematodes in an collector Eppendorf tube. Thus this suspension is poured into the combined tubing construction, the latter is to be hanged in vertical position for 1 hr. During this time nematodes sink down and they are concentrated in the conical part of the Eppendorf collector tube, attached below to the tubing construction (fig. 3, *D*).

4. THE CONCENTRATION OF NEMATODES INTO THE EPPENDORF TUBE WITH AN ADDITIONAL CLEANING OF A SUSPENSION WITH COTTONWOOL FILTER INSERTED IN A TUBING SYSTEM

In some cases all the precautions to keep suspension clean cannot help, and mud may occasionally pass through the filter of the extraction chamber. It makes necessary to clean the already extracted and concentrated nematode suspension additionally. An approach of additional cleaning of a small volume of the concentrated suspension may be done in a tubing construction (fig. 3, *G*). This approach can be used also to extract nematodes directly from crashed bodies of insects avoiding the chamber extraction phase because the total volume of insect does not exceed the tubing construction space.

The extraction procedure is very similar to the procedure described above for the simple sedimentation, with the only exception of input the loose cootonwool filter inside of the tubing and extended extraction time, because nematodes need additional time to pass the filter compared to the simple passive sedimentation in water column under the force of their heavy specific gravity. A loose cottonwool tampon is used as a filter, with a size equal to the half-length of the cylindrical part of a centrifugal tube. This tampon filter is to be inserted into the cut intermediator tube (fig. 3, *F*, *G*). To put the tampon in place it is necessary to remove the cut tube from the tubing construction and attach tightly this intermediator to the collector Eppendorf tube via a 1 cm tubing segment of 8 mm diameter.

A column of two Eppendorf tubes (intermediator and collector) must be filled with water or the physiological saline solution (0.9 % NaCl); the saline solution is to be filled in case of extraction nematodes from crushed insects. Atop a cottonwool tampon to be inserted with a help of needle (fig. 3, *F*). Then the 25 cm main PVC tubing has to be put on the column with the tampon inside; a suspension of nematodes is poured into the total tubing construction from above and the latter is hanged on an upper loop (a cut tube with a hole) for 4 (3—5 hr), exactly as in the previous way of sedimentation without a tampon (fig. 3, *G*). For the mentioned time the actively moving worms pass the cottonwool tampon thus cleaning themselves from mud particles. The suspension becomes clean and suitable both for a vital study under LM or for further preparation: culturing, DNA probe preparation or a fixation to make the collection slides later.

The nematode extraction from crushed insects must be done directly in the tubing with inserted tampon and not in the extraction chamber (fig. 3, *G*). To extend the period of active movement of nematodes through the cottonwool

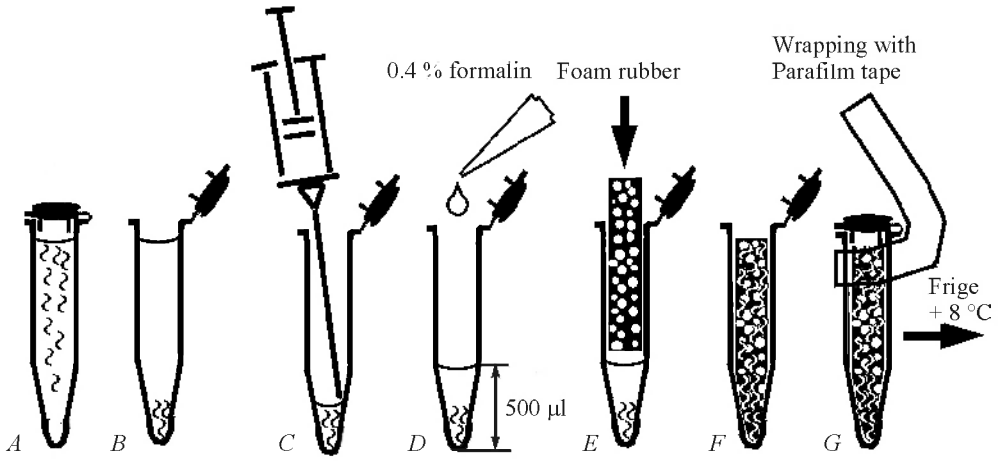

Fig. 4. Preparation of alive nematodes for extended storage.

A — extracted nematodes; *B* — 10 min in vertical position, nematodes settle down in an 1.5 ml Eppendorf tube; *C* — removal of upper layer of water, till 0.5 height of the conical part; *D* — input of 0.4 % formaldehyde solution (until 500 µl, i.e. conical part of the Eppendorf tube), to suppress bacteria multiplication; *E* — placing the foam rubber column to increase the water film surface and air content inside the Eppendorf tube; *F* — nematodes spread in foam rubber; *G* — wrapping the tube head with the Parafilm tape before placing it in a refrigerator for storage at 8 °C.

tampon, water in the extraction-sedimentation tubing is replaced for 0.9 % NaCl solution, which is isotonic to hemocoel. In the same isotonic NaCl solution it is recommended to crash insects before extraction.

5. STORAGE OF LIVE NEMATODES

If nematodes after extraction remain in the Eppendorf tube completely filled with water or 0.9 % NaCl solution they can die within 48 hr even in a refrigerator. It takes place because of the bacteria multiplication leading to a deficiency of oxygen in a tube. Here below some simple expedients are described aimed to increase time to maintain nematodes alive after extraction.

Nematodes are inhabitants of water films. Because of this it is necessary to increase the water film surface and to suppress bacteria multiplication. A 1.5 ml Eppendorf tube remains in vertical position for 5—10 min to allow nematodes to settle down. Then upper water layer has to be removed with a pipette or syringe up to the level of one third of the conical part (0.15 ml) (fig. 4, *A*—*C*). The tube is to be placed into a refrigerator into horizontal position; such position ensures the spread of liquid along the tube. This procedure does increase both air volume and the water film surface of the suspension within the tube. Using this approach it is possible to store the living nematodes in a refrigerator at 8 °C at least for 60 days.

An additional method to store mass quantities of living nematodes extracted from laboratory cultures is the artificial water film surface increase via input of foam rubber pieces in the tube with nematodes. A common foam rubber from a dishwashing sponge is cut to pieces. As in the above-described method, a tube with extracted nematodes is kept in a vertical position for 10 min to sink nema-

todes down, then it is necessary to remove the upper water layer with a syringe leaving 0.5 ml of water (the conical part of the Eppendorf tube). Then the foam rubber pieces are to be put inside allowing them to expand in the tube and soak excess water. Using this method nematodes may be kept alive in the refrigerator at 8 °C for at least 2 months; however, nematodes are gradually dying while maintaining (fig. 4).

During storage, a share of dying nematodes extracted from laboratory cultures may be significant if the total number of nematodes is large; it is caused by the prompt change of the microenviroment conditions compared to the culture media and because of a lack of a food source. The largest adults die first, their deaths lead to decay and consequently multiplication of bacteria, thus causing oxygen deficiency. To suppress bacteria multiplication it is necessary to use very low concentration of formaldehyde, which kills bacteria but keeps nematodes alive. By experience, such concentration does equal 0.2—0.4 % formaldehyde solution in water. The nematode extraction and their concentration are processed as described above. The Eppendorf tube is kept in vertical position for 5 min and then upper water layer is to be removed leaving 0.25 ml of water (a half of the conical part of the centrifugal tube). Then the 0.4% formaldehyde solution is to be added (tenfold diluted 4 % formalin fixative, commonly used) until 0.5 ml total volume (fig. 4, *D*). Then pieces of foam rubber are to be added to fill the Eppendorf tube with the nematode suspension, allowing them to expand and soak excess water (fig. 4, *E*, *F*). A tube has to be marked with a sticky label and its cap is bandaged with a Parafilm band or with elastic stretch wrap (fig. 4, *A*—*G*). Using this method it is possible to maintain nematodes alive in a refrigerator for 6 months at 8 °C . Such long time of storage is economically expedient because it can save a lot of working time and expendable materials, which are necessary to maintain experimental nematode isolates and genetic strains. Pre-adult stages and dauer juveniles of nematodes survive mainly in this way of storage; they may be easily multiplied in laboratory cultures.

DISCUSSION

The modification of extraction method that is described above was tested during three years on 3000 samples of wood, plant tissues and soil. This technique is enough reliable and efficient according to results. The proposed modification of the Baermann funnel completely excludes a funnel itself or any sloping surfaces, but the fibrous cottonwool filter remains the basic element of dynamic division of the actively moving nematodes from immovable substratum particles. The principle of separation is the same: dynamic passage of narrow actively moving worms via pores of a filter with simultaneous sedimentation of nematodes under the force of heavy specific gravity of their bodies.

The earlier not analyzed principle of the action of the routine empiric Baermann's technique (fig. 1) in a new modification is illustrated here; the simplification usable in field condition is proposed with a simultaneous increase of its efficiency: a share of nematodes passing the filter in clean water increases because all possible contacts of the sticky nematode bodies with sloping surfaces of the extraction chamber are minimized; the new technique strictly follows the principle that nematodes must sink in vertical water column with abrupt walls.

In the classical Baermann version the equipment was made from glass, rubber tube and metal, thus it was voluminous, heavy and fragile which made its usage in field conditions not user-friendly. In the proposed here modification all compartments are very light-weighted, compact in transportation, simple, reliable in use, and cheap; the compartments (tubing, cotton pads and plastic cups) can be easily bought in a supermarket and then combined into the extraction chamber and tubing system. The equipment may be use as disposable because it very cheap, but after washing the laboratory set may be used several times, to avoid environment pollution.

An additional advantage of the method is the possibility to use it by non-professionals, even by people without biological background or knowledge, e. g. it may be used by the technical staff of the Polar expeditions because the technique allows the «blind» use without obligatory LM study of extracted nematode suspension just on a place of survey.

In addition, the efficient method to maintain nematodes alive for a long time without re-culturing is proposed here. It is necessary to create the water film microenvironment in a tube of storage, removing excess water and adding pieces of moist foam rubber. To increase the time of storage up to several months it is necessary to suppress the bacteria multiplication in storage tube adding 0.2—0.4 % formaldehyde solution: it does kill bacteria but is not dangerous to nematodes.

Fixation of extracted nematodes for morphological study. The best way to fix nematodes for future morphological study under LM is a method of hot fixation proposed by Seinhorst (1959). I developed a modification of this way of fixation exactly for field conditions far away from laboratory; the protocol combines both a fixation procedure and a method of preparation of permanent collection slides in one description. It will be published soon.

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