Growth of Two Species of Collembola (Apterygota: Insecta) in Three Different Culture Methods

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The recent emphasis on nutrient cycling as a means of quantifying ecosystem process indicates the importance of micro-arthropods in the soil /WAIDE et al., 1974/. Various studies on soil arthropods have shown that Collembola represents one of the most abundant groups of soil arthropods and it may play a dominant role in soil formation and litter decomposition. For the clear understanding of their role in the decomposition process, it is necessary to know the quantitative aspects of the population of individual species of Collembola by analysing their dynamics, and to study the biology, growth, age structure and fecundity of individual species of Collembola under controlled conditions. To study the growth pattern these organisms are usually cultured in the substrate of plaster of paris and charcoal. PANDA /1984/, while using this substrate, also used sand and plant litter as substrates to study the growth pattern of two species of Collembola /Cryptopygus thermophillus and Lipidocyrtus caerulicornis/ and concluded that the sand was the most suitable substrate for the above two species. So, this investigation has attempted to study the growth pattern of two other species of Collembola, such as Cyphoderus javanus and Entomobrya atrocincta in the three culture methods and to draw a conclusion whether or not any significant differences existed between the culture methods.

Materials and methods

A mixture of plaster of paris and charcoal was widely used as a base on which collembola was cultured. There have been considerable variations in the composition of the plaster charcoal base. BOOTH /1983/ showed the effects of plaster charcoal substrate variation on the growth and fecundity of F. candida. Charcoal helps to absorb any harmful volatiles from the plaster or metabolites from the cultured animals. It also provides a rough visual guide to the degree of saturation of the plaster. Besides this standard method some amount of fine sand and plant litter can also provide a suitable substrate to culture the spring-tails. PANDA /1984/ studied the growth of two species of collembola i.e. C. thermophilus Axelson and L. caerulicornis Bonet in three different culture methods. He found that the sand culture method was most suitable followed by the litter culture method, whereas the plaster-charcoal method

was least preferred. The detailed descriptions of the three culture methods are described as under.

Preparation of culture dishes

Plaster-charcoal method. - In this method, collembola were cultured on a mixture of 9 parts of plaster of paris and 1 part of activated charcoal without adding any fungicide. The dry plaster of paris and activated charcoal were mixed initially 9:1 by volume, respectively. Distilled water was added to the above mixture to make a pouring slurry which was poured into plastic culture vessels /Diameter 7.5 cm, height 8 cm/ up to a height of 1 cm. After this the surface was brushed and washed with distilled water. The newly prepared plaster charcoal vessels were allowed to dry up completely because it was noticed that premature use of the base resulted in a high initial mortality of the animals. Distilled water was added nearly to the saturation point before introducing Collembola into the culture vessels.

Sand culture method. - Acid washed sand was used to rear collembola. Fine granules of sand particles were washed with diluted sulphuric acid and then were washed thoroughly with distilled water to remove the acid from the sand. The sand particles were dried up either in the oven or in the air. The bottom of the cylindrical plastic culture vessels were filled with the acid washed sand up to a height of 1 cm. Then distilled water was added drop-wise to the sand substratum up to the saturation point before introducing collembola. There was no addition of any fungicide to the culture.

Litter culture method. - Plant litter was used as the substrate on which collembola were reared. Some dried fallen leaves of Mangifera indica Linn were collected, washed with water thoroughly to remove the last trace of other particles on it. Then the leaves were dried in oven and thus sterilised. The sterilised leaves were grinded to powder. The dried plant litter powder was taken in individual culture jars to form a substratum of 1 cm height. Distilled water was added drop-wise nearly to the saturation point. The culture was maintained without the application of any fungicide and without adding any type of food.

Experimental animals

Two species of collembola Cyphoderus javanus. Borner-Zess and Entomobrya atrocincta Bonet were used as experimental animals.

Food supply

Dried yeast was supplied as food to the plaster-charcoal and sand culture dishes. About 50 mg of dried yeast pellets were provided each time at an interval of 10 days, keeping the medium completely saturated with a high relative humidity of 90-100% in distilled water. The older food pellets were usually removed from the substrate surface in the culture dishes before the addition of new pellets. Food was not supplied to the litter culture vessels as this was considered as self-feeding medium.

Culture experiment

The aforesaid two species of collembola for this investigation were collected from the soil under decaying logs of wood. Only the adult specimens were isolated and transferred to the culture vessels. The culture containers were closed with finely meshed cloth by tight rubber band with the container lid over it.

At the outset, 30 plastic culture containers /diameter 7.5 cm and height 8 cm/ were used out of which 10 contained plaster charcoal substrate, 10 sand substrate and 10 with litter substrate. Ten /five male and five female/ healthy adult individuals belonging to the species C. javanus were released to five culture dishes of the three different substrates.

Similarly, then /five male and five female/ healthy adult individuals of E. atrocincta were released into five culture containers of three different substrates. The experiment started on 2nd May, 1987 and continued up to 9th October, 1987. All the culture containers were maintained undisturbed at room temperature that varied from 18 ^{o}C to 31 ^{o}C and were kept in darkness in a wooden cupboard in the laboratory. The containers were only brought outside at the time of examination. At an interval of 10 days, the culture dishes were examined to find out the number of collembola present.

Results

The population number of Cyphoderus javanus and Entomobrya atrocincta in three different cultures at different days have been shown in Fig. 1. In all the culture methods, both species showed typical sigmoid growth curves. At the beginning, the rate of change in population was very slow, which was followed by a rapid growth period after which no significant change occurred. The species, C. javanus in the litter culture showed very slow growth up to the 70th day, after which there was a rapid growth up to the 120th day, and then the population remained almost constant. In sand and plaster charcoal cultures the species showed a very slow change up to the 100th day. Then there was a small increase in the rate of change in population and after the 140th day the population number remained almost constant. The species, Entomobrya atrocincta in the litter culture showed very slow growth up to the 80th day after which there was rapid growth up to the 130th day and then the population number remained almost constant. In the sand culture, this species showed a very slow change up to the 110th day after which there was some increase in the growth up to the 150th day, where the population number stabilised. The species in the plaster of paris charcoal culture showed a very slow change throughout the study period and reached the stabilisation period at the 140th day.

Table 1 gives account of the ANOVA /analysis of variance/ for C. javanus and E. atrocincta, respectively. From the ANOVA tables, it was observed that there were significant variations between the cultures and also between the days in both species /$P < 0.05$/.

The mean population of the species, C. javanus in the litter, sand and plaster charcoal culture were 53, 34 and 27, respectively. The respective values for the species E. atrocincta were 39, 23 and 21. The t-test was applied to calculate the critical difference to ascertain the significant differences between the individual cultures and to arrange the culture methods in order of preference basing upon their efficiency. The details of the critical difference test for both species have been given in Table 2. For both species it was convincingly concluded that the litter culture was the most suitable method over the other two. For the species, Entomobrya atrocincta

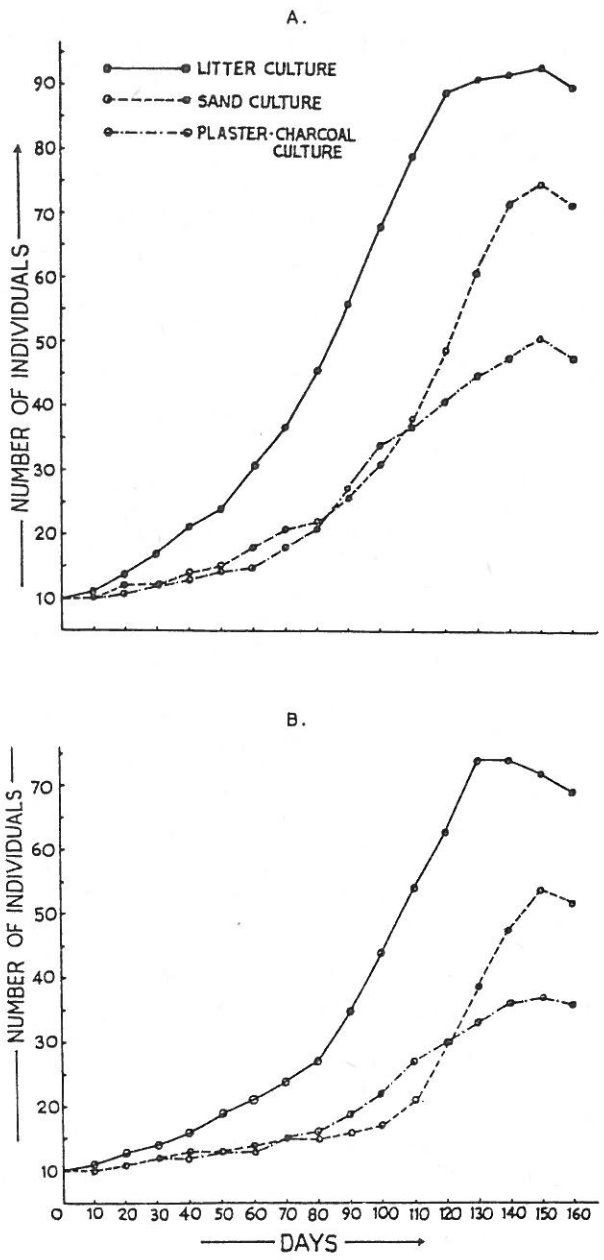

Fig. 1
Total population of Cyphoderus javanus /A/ and Entomobrya atrocincta /B/, at different days in three different culture methods during the study period

Table 1
ANOVA table for Cyphoderus javanus and Entombrya atrocinota

Source of variation	Degree of freedom	Sum of squares	Mean squares	F ratio
		Cyphoderus javanus		
Between cultures	2	5793.16	2899.08	31.04
Between days intervals	15	24617.48	1641.16	17.57
Error	30	2801.83	93.39	-
Total	47	33217.47		
		Entombrya atrocinota		
Between cultures	2	3033.16	1516.58	23.47
Between days interval	15	12164.58	810.97	12.01
Error	30	2024.16	67.47	
Total	47	17221.90		

Table 2
Critical difference /CD/ for the species Cyphoderus javanus and Entomobrya atrocincta

Cyphoderus javanus	Entomobrya atrocincta
CD = 5.79	CD = 4.92
$\bar{T}_1 - \bar{T}_2 = 19.37 > 5.79$	$\bar{T}_1 - \bar{T}_2 = 15.62 > 4.92$
$\bar{T}_2 - \bar{T}_3 = 6.5 > 5.79$	$\bar{T}_2 - \bar{T}_3 = 2.25 < 4.92$

Remarks: $\bar{T}_1$ = Mean population of collembola in litter culture; $\bar{T}_2$ = Mean population of collembola in sand culture; $\bar{T}_3$ = Mean population of collembola in plaster-charcoal culture.

there was no significant difference between the sand and plaster of paris--charcoal culture. But for the species, Cyphoderus javanus there was also significant difference between sand culture and plaster of paris charcoal culture.

Discussion

The demographic studies of collembola from the laboratory culture reveal that these organisms have an exceedingly high intrinsic capacity to increase /GREGOIRE-WIBO and SNIDER, 1977/. The population also stabilises at a point. In this investigation, too, both species have shown tendency for stabilising at a particular point after the growth period. There are various explanations

by the earlier workers to understand the actual mechanism of the equilibrium stage. SNIDER /1983/ - in his experiment with Onychiurus folsomi under laboratory condition at constant temperature - observed that the egg production in low number reared culture was increased at least four-fold over that in mass culture. After initial fecundity a gradual decline towards the 4th week was succeeded by an increase to approximately the 36th week. GREEN /1964/ found that fecundity was reduced in crowded culture of Folsomia candida. Oviposition was inhibited by contact with individuals attempting to lay eggs and those searching for food. Yeast or bacteria apparently can produce a contaminant in progressively aging culture, resulting in substantial inhibition of egg production in the cultures of Sinella curviseta /WALDRUF, 1971/. The influence of crowding, yeast and bacteria have decreased the egg laying in mass cultures of O. folsomi. The crowding effect could be minimized if individuals were sampled from the culture container for experimental use at regular intervals.

Similarly, the accumulation of excretory products might contaminate the surface of cultures and inhibit oviposition. CHRISTIANSEN /1967/ using several species of collembola, found depression of reproduction in cultures after 200 days. SNIDER /1973/ transferred his experimental animals to fresh cultures whenever the substrate became badly contaminated. In this investigation also, efforts were made to remove the excretory products and old food pellets at regular intervals. Thus, the stabilisation of growth curves were mainly due to the mass effect. Similar results had also been encountered when HUTSON /1978/ experimented with F. candida to study the effects of variation of the plaster-charcoal culture method. He reported that the number of eggs produced in high density cultures were generally far fewer than those expected on the basis of result from the low density cultures and he also suggested an adverse effect due to population density. GREEN /1964/ - also working with F. candida - reported that the optimum surface area per individual for oviposition in mass cultures was about 1.2 cm^2 and this figure was supported by CHRISTIANSEN /1967/. In the present investigation for both species the surface area in the culture dishes was 44.19 cm^2, thereby not allowing sufficient space for oviposition. So it could be concluded that if the substrate area would be increased it might be possible to get more individuals from a single culture.

The second object of this investigation was to assess the most suitable and efficient culture method among the three. It was evident that the population growth of both species was highest in the litter culture. Plaster of Paris charcoal was the least preferred. PANDA /1984/ had made a comparision of these three culture methods using C. thermophilus and L. caerulicornis as experimental animals. For these two species, he convincingly found sand culture as the best method, which was followed by litter and plaster-charcoal method.

Litter and sand culture methods were more economical and the preparation of both cultures were less time-consuming as compared to the conventional method.

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