

UNIVERSITY OF SWAZILAND

MAIN EXAMINATION PAPER 2016

TITLE OF PAPER : **BIOLOGICAL RESEARCH TECHNIQUES**

COURSE CODE : **BIO602 / ERM610**

TIME ALLOWED : **THREE HOURS**

INSTRUCTIONS : **1. THIS PAPER HAS FIVE (5) QUESTIONS
2. ANSWER QUESTION 1 (COMPULSORY)
PLUS ANY TWO (2) OTHER QUESTIONS
3. WHEREVER POSSIBLE ILLUSTRATE
YOUR ANSWERS WITH LARGE
CLEARLY LABELLED DIAGRAMS**

SPECIAL REQUIREMENTS: **NONE**

**THIS PAPER IS NOT TO BE OPENED UNTIL PERMISSION HAS BEEN
GRANTED BY THE INVIGILATORS**

Question 1

A journal article is provided. Read the article and write your own abstract. Your abstract should be properly formatted, ensuring that it includes all critical information.
[30 marks]

Question 2

Discuss the application of molecular techniques in exploration and preservation of plant genetic resources.
[30 marks]

Question 3

Examine cryopreservation techniques as applied in herbaceous species and recalcitrant seeds.
[30 marks]

Question 4

Describe how one could isolate and quantify bacterial population from soil.
[30 marks]

Question 5

Describe and discuss staining theory in histology. Your discussion should be guided by (but not limited to) the following questions:

- i. Why do any of the tissue components stain?
- ii. Why do stained components remain stained?
- iii. Why are all components not stained?

[30 marks]



**INTERSPECIFIC COMPETITION BETWEEN *ANISOPTEROMALUS CALANDRAE*
(HOWARD) (PTEROMALIDAE) AND *DINARMUS BASALIS* (ROND.)
(PTEROMALIDAE) ON *CALLOSOBRUCHUS CHINENSIS* (L.)**

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Introduction

Anisopteromalus calandrae (Howard) and *Dinarmus basalis* (Rond.) are cosmopolitan, solitary and ectoparasitic parasitoids of and potential parasitoid of different stored grains and pulse beetles (Ghani and Sweetman 1955, Ahmed 1991, Markham et al. 1994, Boucek and Rondani 1974, Heong 1981, Okamoto 1971).

It may be stated that there exists two major classes of competition among individuals in or on the host. They are gregarious and solitary ectoparasitoids (Samways 1971). The ovipositing parasitoid females avoid superparasitism when the hosts are adequate. But, when the hosts are not plentiful, then superparasitism often occurs. Due to superparasitism, competition between the larvae of different parasitoid species occurs and only one parasitoid larva survives and emerges out as an adult.

In solitary parasitoids, multiple larvae present in the same host larvae or pupae are eliminated by the competitive older larvae generally. When a female reencounters the host on which she oviposited before and deposits egg(s) onto it, 'self superparasitism' occurs. But, when a female encounters a host parasitized by another conspecific female(s) and lay(s) on it, 'conspecific superparasitism' occurs (Bakker et al. 1985). Many workers studied on the mechanism of larval competition of endoparasitoid species (Chow and Mackauer 1984).

The effects of interspecific competition between *A. calandrae* and *D. basalis* on their parasitism and progeny production were investigated in the present study.

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Materials and Methods

A. calandiae and *D. basalis* obtained from the stock of mass culture on *C. chinensis* infesting lentil maintained in the Pest Control Section, Applied Zoology Research Division, BCSIR Laboratories, Rajshahi since ten years. The experiments were conducted at $30 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ rh. Host containing seeds of lentil of 15-21 days old were used as 4th instar larvae, prepuae and pupae of *C. chinensis* throughout the study period.

To determine the effects of interspecific competition between *A. calandiae* and *D. basalis* on their progeny production, 20 4th instar larvae, prepupae and pupae containing lentil seeds parasitised by *A. calandiae* were mixed with the same number of *C. chinensis* parasitised by *D. basalis* (i.e., a total number of 40 host containing seeds of both species) were confined in different petri dishes (15 cm diam.) and kept separately in plastic containers (15X25 cm) and run at $30 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ rh in an incubator.

One mated female of *A. calandiae* and *D. basalis* were released in separate petri dishes for egg laying upto 24 hr and then removed and kept in an incubator at the same temperature until emergence. The number of adults emerged from each host species in different replications were counted and compared. Subsequently, the sizes of both the adult parasitoids were also recorded. The progeny produced at 48, 72, 96 and 120 h duration were carried out in the same manner as described above but a fixed number of 80, 120, 160 and 200 host containing lentil seeds of *A. calandiae* and *D. basalis* were supplied at aforesaid duration respectively.

To determine the parasitism of *A. calandiae* and *D. basalis* as a result of interspecific competition, the experiments were conducted on the above manner. But the highest and lowest numbers of *A. calandiae* and *D. basalis* adults emerged from each replication were observed and recorded. The dead hosts inside kernels and the adult *C. chinensis* were also recorded. All the experiments were also run at the same temperature and rh.

Results and Discussion

The results of interspecific larval competition between *A. calandiae* and *D. basalis* on *C. chinensis* in mated condition is presented in Table 1. It is striking to note that the number of progeny production is always lower in higher duration of 48, 72, 96 and 120 h compared to the number of progeny produced in 24 h.

From the Table 1, it is also obvious that *D. basalis* progeny is always higher than *A. calandiae* progeny in all durations. So, it can be concluded that *D. basalis* is a dominant parasitoid to establish its progeny in the interspecific competition with *A. calandiae*. A mean total number of 16.9 ± 2.76 and 56.7 ± 9.86 progeny of both the parasitoids were produced at 24 and 120 h duration respectively. According to Wai and Fuji (1990), during intra specific larval competition among ectoparasitic wasps, *D. basalis*, *A. calandiae* and *Heterospilus prosopididis* (Viereck) (Hymenoptera: Pteromalidae) for their host, *C. chinensis* it was revealed that 83 to 92 wasp offspring could emerge as adults from 100 hosts in all three species. They opined that 16-17 day old host in terms of amount of resources can support the development of at least 2 larvae of *A. calandiae*. Among these parasitoids, *D. basalis* highest progeny of 86 offspring in 1 egg-host condition observed and *A. calandiae* produced 81 progeny at the same condition. It is evident from Table 1, sex ratios were 1.76 and 2.75 in *A. calandiae* and *D. basalis* in 24 hr duration respectively.

Table 1. Results of intra specific larval competition between *A. calandiae* and *D. basalis* on the progeny production in different intervals from individual mated female.*

Duration (h)	Mean no. of wasps emerged (Mean ± S.D.)	Mean no. of parasitoids emerged from individual mated female		Sex ratio (Female to male) <i>A. calandiae</i> <i>D. basalis</i>	
		<i>A. calandiae</i>	<i>D. basalis</i>		
24	16.9 ± 2.76	7.8 ± 1.99	9.1 ± 3.66	1.76	2.75
48	20.7 ± 5.69	6.8 ± 3.85	13.9 ± 3.38	1.27	1.50
72	31.1 ± 7.47	10.3 ± 6.56	20.8 ± 2.44	2.49	1.65
96	38.6 ± 5.87	15.5 ± 7.26	23.1 ± 5.98	1.06	1.75
120	56.7 ± 9.86	24.8 ± 13.05	31.9 ± 19.58	1.42	2.54

* Data based on 15 observations.

Table 2. Mean per cent parasitism of *C. chinensis* by *A. calandiae* and *D. basalis* confined at different duration. *

Duration (h)	No. of host infested seeds supplied	Total % parasitism by the two parasitoids	Individual parasitism (%)			
			Highest		Lowest	
			<i>A. calandiae</i>	<i>D. basalis</i>	<i>A. calandiae</i>	<i>D. basalis</i>
24	40	30.0	25.0	50.0	12.50	32.50
48	80	16.56	15.0	33.75	2.5	15.0
72	120	18.53	18.33	36.66	3.33	15.83
96	160	13.75	16.25	30.0	1.88	6.87
120	200	14.75	21.0	32.0	2.0	4.0

* Data based on 15 observations.

The mean per cent parasitization of *C. chinensis* by *A. calandiae* and *D. basalis* is summarized in Table 2. It is apparent that individual parasitism was gradually decreasing with the increase of host numbers and duration of egg laying by both the parasitoids. This observation is in accordance with the view of Ahmed and Kabir (2002) in *A. calandiae* with its host, *Rhyzopertha dominica*. In the present study, it was observed that the older parasitoid larvae of either *A. calandiae* or *D. basalis* usually survived to adults. Thus, super parasitism might be rather disadvantageous and selected against in nature because of the complete wastage of egg (s) for female parent in subsequent oviposition. So far, many studies have been conducted on the mechanism to avoid super parasitism (Ables et al. 1981, Hubbard et al. 1987).

However, it may be concluded that *A. calandrae* and *D. basalis* avoided super parasitism during inter specific competition for their host, *C. chinensis* when confined to different host numbers at different durations.

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