zonium salt, was measured at 500 nm using a Beckman DK 2A Ratio Recording Spectrophotometer. The optical density values of the experimental (with methyl paraoxon) and control (without methyl paraoxon) samples were compared and the per cent cholinesterase inhibition was calculated.

Results and Discussion

For assaying the cholinesterase activity of the human serum, the amount of organic solvent in the reaction mixture was kept to the minimum. For this reason the methyl paraoxon was prepared in distilled water, as its solubility in water is more than that of parathion, whereas 2-naphthyl acetate was added in 0·01 ml of acetone as this volume of acetone did not cause any turbidity in the reaction mixture. Addition of 0·04 ml of acetone caused slight turbidity. The colour formed due to coupling reaction described early is stable indefinitely.

The initial velocity of the enzyme reaction was studied with respect to time, substrate and these factors were appropriately chosen for assaying the cholinesterase activity. The results show that per cent cholinesterase inhibition increased with the increase in the methyl paraoxon concentration (Table 1).

TABLE I

Effect of methyl paraoxon on human serum cholinesterase activity

No. of observations in each case—12.

Concentration of methyl paraoxon μgm/ml	Per cent ChE inhibition
0·5	+10± 3
1	−18± 5
2	−43± 5
3	−54± 3
4	−63±11
5	−67±11
6	−75±15
7	−74± 4

Enzyme activity 100% = 29·2 μm. 2-naphthyl-acetate/ml of serum/hr.

+ % Activation over control; − % Inhibition over control; ± Standard Deviation of mean.

However below 1 μgm. ($4·2 \times 10^{-6}$ mole) of methyl paraoxon, the cholinesterase activity showed a slight increase (Table 1)) The enzyme activation at a very low concentration of methyl paraoxon, *i.e.*, 0·5 μgm. ($2·1 \times 10^{-6}$ mole) can be corroborated with the results reported earlier[7] wherein similar cholinesterase activation at concentrations of 5×10^{-5} to 5×10^{-7} mole of methyl paraoxon in the liver of monkey was observed. The cause of the activation at such low concentrations has not been clearly explained[7]. The cholinesterase inhibition showed variation upto 15% from the standard mean values in a few observations. This might be attributed to the variation in the cholinesterase levels of the individuals themselves. For example, repeated tests over short term and long term periods ranging from one week to five years on humans have shown variation in the cholinesterase activity levels from 7 to 11·3% as observed by several investigators[8–11].

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A SIMPLE MEDIUM TO DETECT L-ASPARAGINASE POSITIVE BACTERIA

L-ASPARAGINASE (L-asparagine amidohydrolase E.C. 3.5.1.1.) is known to possess anti-leukaemic properties in animals[1] and in human beings[2]. In order to meet the requirements for clinical use, the enzyme has to be produced in larger quantities. This prompted many researchers to screen large numbers of bacteria for L-asparaginase activity[3–7].

TABLE I

Comparison of the conventional method to detect L-asparaginase activity with the media formulated in the study

Sl. No.	Cul. No.	A*	B**	Activity IU/mg protein***	Sl. No.	Cul. No.	A	B	Activity IU/mg protein
1	41	+	++	0·05	32	408	+	++	0·04
2	93	+	+	0·03	33	462	+	+++	0·03
3	102	+	++	0·01	34	507	++	++	0·08
4	105	−	−	−	35	516	+	++	0·02
5	109	−	−	−	36	521	+	++	0·05
6	124	+	++	0·06	37	528	+	+++	0·06
7	152	−	−	−	38	691	+	++	0·05
8	153	++	+++	0·08	39	700	−	−	−
9	166	+	+	0·08	40	705	+	+	0·03
10	167	+	++	0·06	41	708	+	++	0·11
11	170	+	++	0·03	42	709	+	+	0·08
12	183	−	−	−	43	718	−	−	−
13	192	+	++	0·03	44	735	+	++	0·14
14	194	−	−	−	45	736	−	−	−
15	212	+	+	0·05	46	737	−	−	−
16	216	+	++	0·06	47	742	+	++	0·16
17	217	+	+	0·05	48	749	−	−	−
18	227	+	+	0·04	49	751	−	−	−
19	228	+	++	0·15	50	757	+	++	0·15
20	234	+	+	0·09	51	758	−	−	−
21	241	+	++	0·02	52	819	−	−	−
22	243	+	++	0·06	53	826	+	++	0·03
23	244	+	+++	0·16	54	834	−	−	−
24	265	−	−	−	55	838	−	−	−
25	281	−	−	−	56	848	+	+++	0·07
26	299	+	++	0·04	57	849	−	−	−
27	322	++	+	0·03	58	857	+	+++	0·18
28	340	+	++	0·11	59	852	+	+	0·05
29	342	+	++	0·07	60	885	+	++	0·14
30	349	+	+	0·02	61	886	−	−	−
31	364	++	++	0·07	62	888	−	−	−

* Medium A; ** Medium B; *** L-asparaginase activity by the conventional method.
+, ++, +++, − Intensity of the colour change in the media.

All the authors have used conventional methods to detect the activity of this enzyme in the cultures. Generally it involves the growth of the organism, harvesting the cells, preparation of cell suspensions and then the assay. This is time consuming and tedious. A medium which will enable one to select L-asparaginase positive cultures very easily has been described here.

The cultures used in this study were taken from the culture collections of the CAS in Marine Biology, Parangipettai. Before use, they were subcultured onto ZoBell's medium to get fresh colonies.

The media used were as follows :

Medium A : Peptone—1 g; Sodium chloride—5 g; KH_2PO_4—2 g; L-asparagine—20 g; phenol red (2 g/l aqueous solution) 6 ml; Agar—20 g; distilled water 1 l; pH 7·00.

Medium B as above except agar-agar.

The media were sterilized at 121° C for 15 min. in an autoclave. Control media were also included in which the substrate L-asparagine was omitted. L-asparaginase converts L-asparagine into aspartic acid and ammonia. This can easily be detected by the change in the pH of the medium using phenol red.

The media, inoculated with fresh cultures, were incubated at 28 ± 1° C for 24 hrs. The change in the colour (pink) of the medium was noted in the presence of L-asparaginase positive cultures while no such colour change could be seen with negative cultures.

All the cultures were screened by the conventional method[8] to confirm the L-asparaginase activity as above.

Results obtained in the present study are shown in Table I. L-asparaginase activity was absent in 34% of the total cultures examined in the present study. It is clear that the cultures which exhibit the enzyme activity by the conventional method were also giving positive reactions in the media used. None of the control media showed any change in the colour of the medium, though growth was observed. The colour change was noticed earlier in the liquid medium as compared to the slants. This may be due to the rapid growth of the organism in the medium B. The intensity of the colour of the medium deepens as the incubation continued and remained unaltered after 20 hrs. of growth.

It is also quite clear from the table that the enzyme activity has no relationship with the degree of the colour intensity of the medium. This is due to the change in the conditions of the assay mixture.

The present study thus clearly indicates that medium A or medium B can safely be used to detect quickly L-asparaginase positive cultures both for the screening programme and also for taxonomical studies. There are some reports[6,9,10] which show that this enzyme activity could be used in taxonomical studies of microorganisms.

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EFFECT OF JUVENILE HORMONE ANALOGUE ZR-515 ON THE LAST INSTAR NYMPHS OF *DYSDERCUS CINGULATUS* FABR. (PYRRHOCORIDAE : HETEROPTERA)

Introduction

JUVENILE hormone analogue (JHA) is an effective growth regulator in insects Wyatt[7]. Insect hormones and their synthetic analogues are considered to be very promising because they show high biological activity, low mammalian toxicity and short environmental persistence. In the present investigation the juvenile hormone analogue ZR–515 was tested on the last instar nymphs of *Dysdercus cingulatus* Fabr. in different concentrations.

Materials and Methods

The test insects used in this experiment were from a pure culture maintained in the laboratory at 28±2° C reared on soaked cotton seeds. Various concentrations of ZR–515–Altosid [isopropyl (2E, 4E)–11–methoxy–3,7,11–trimethyl–2–4–dodecadienoate; Zoecon Corporation, U.S.A.] were applied topically to freshly moulted last instar nymphs. Two concentrations 0·25 and 0·5% of JHA were prepared in 1% acetone solution; for control only 1% acetone was used. Each of these concentrations was applied individually on the dorsal surface with the aid of a micropipette (2 µl per insect). Sixty nymphs were