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The changes in the hatchability & survival of Egg & Larvae of sheep nematode after exposure to Ultraviolet radiation

التغيرات في قابلية الفقس وبقاء بيوض ويرقات ديدان الأغنام الأسطوانية عقب التعرض للأشعة فوق البنفسجية

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Abstract :

The effect of ultraviolet (UV) radiation on the hatchability & the activity (survival) of sheep gastrointestinal nematode;Haemonchus contortus, trichostrongylus colubriformis , Oesophagostomum columbianum and Strongyloides papillosus eggs & Larvae were examined. The hatchability decreased with increasing exposure to radiation. The difference in hatchability of eggs irradiated for 15.30 & 60 minutes were highly significant ($P> 0.01$ $d\alpha = 3.08, 3.25$ and 3.76 respectively) compared with the hatchability of the non-irradiated eggs. The life span of irradiated was shortened , only (19%) of those expose to UV radiation 60 minutes survive for 2 days as against (100%) survival rate in the non-irradiated larvae . Batches of nematode larvae (L1) were irradiated with ultraviolet light for varying time interval to determine the influence of radiation on the transmission potential of the irradiated larvae. There was a decreased in the survival rate of the hatched free-swimming larvae that corresponded with the increasing radiation exposure time .

الخلاصة:

تم قياس تأثير الأشعة فوق البنفسجية على قابلية الفقس وفعالية (بقاء) بيوض و يرقات ديدان معدة وأمعاء الأغنام الأسطوانية Haemonchus contortus، Trichostrongylus colubriformis، Oesophagostomum columbianum و Strongyloides papillosus . لقد انخفضت قابلية الفقس بزيادة التعرض للإشعاع ، إذا اظهر التباين في قابلية فقس البيوض المشعة لـ (١٥) ، (٣٠) ، (٦٠) دقيقة فروقاً معنوية عالية ($P <$٠.٠١ $d\alpha = 3.08 , 3.25 , 3.76$ على التوالي مقارنة بقابلية الفقس في البيوض غير المشعة . وتقلص عمر البقاء لليرقات المشعة ، حيث إن (١٩%) فقط من التي تعرضت لـ (٦٠) دقيقة إشعاع استطاعت البقاء حية لمدة يومان فقط ، بالمقابل كان معدل البقاء لليرقات غير المشعة (١٠٠%) كما تم تشعيع مجاميع من اليرقات (L1) بالأشعة فوق البنفسجية في أوقات متباينة لمعرفة تأثير التشعيع على إمكانية الانتقال لليرقات المشعة ، إذ لوحظ انخفاض في معدل بقاء اليرقات السابحة عقب الفقس بزيادة وقت التعرض للإشعاع .

Introduction :

The influence of several factors such as PH, temperature, light , salinity and redox potential on the hatchability of nematode eggs have been reported previously . The influence of radiation on a variety of living organisms has been observed to vary from partial to total interference with normal development process (Stowen , 1968 ; Farvar & Cember , 1969 ; weile & Mertens, 2007).The continues & significant increase in trace gases which is responsible for a gradual erosion of the stratospheric Ozone layer which are expected to lead to an increase of ultraviolet (UV) radiation at the earth surface (Nairobi declaration, 1990). An Argumentation of incident biologically effective radiation wave length band (U.V.B) between 290 and 325mm could be a serious risk factor in the future (Litchenberg & Sadum , 1963 ;Prah & James , 1977).The present study aimed at determining the possible influence of change in UV radiation intensities base on length of exposure on the hatching of nematode eggs as well as the survival rate of the larvae obtained from the irradiated larvae based on expected changes in radiation intensities associated with the mean annual global

temperature increase.

Materials & Methods:

- **Nematode egg recovery technique**:

10-15gm of faces were suspended in water and removal of organic debris have been done by filtration through sieves (1mm and 100µm) the eggs being collected on a 20µm sieve . The eggs were further separated from organic debris by centrifugation in magnesium sulphate (density 1.16) for (5) minute at 1000g. The supernatant was filtered through 100µm & 60µm sieves and the eggs were washed in water and collected on a 20µm sieve , according to technique previously described by Hurbert and Kerbouef (1992).

- **Egg suspension :**

The concentration of eggs was in five 50µl samples and adjusted to 1200-1300 egg ml . Bacteria (non pathogenic serotypes of E. coli) are necessary for the development of the nematode larvae and must be added to the medium. The egg suspension was dilute with filtrate from the first step of egg extraction , which had been centrifuged for (5)minutes at 1000g to eliminate organic debris. To avoid the proliferation of fungi , 5µg of amphotericin B (Fungizon ND) was added per ml of egg suspension. (Keeling, 1960;Micheals & Kean , 1969).

- **Nutritive medium :**

Hubert & Kerbouef (1992) describe nutritive medium was compose of Earls' balance salt solution plus yeast extract (Difco laboratories) diluted in saline solution (1g of yeast extract / 90ml of saline solution) in the proportion 1:9 volume to volume of egg suspension .

- **Hatchability test:**

100µl of egg suspension containing approximately (100) eggs was added to each of the six Petri dishes (90mm diameter), eggs were counted by use of the modified Macmaster method (Whitlock , 1949) Five of those were exposed to UV radiation from a lamp source emitting a wave length of (2.54)mm from a distance of (560)mm from the base of the containers to the radiation source. Radiation exposure of the egg batches were for 0,1,5,15,30 & 60 minutes respectively.20ml of nutritive medium was later added to egg suspension and put in an incubator at (27 ° C). the first stage of larvae were obtained two days later , At this time , the parasites were diagnosed and counted by the method that mentioned by (Theodoride ,1964) . The counting is done for another (6 day) to determine the survival rate of the larvae .By this time the larvae has developed to third stage larvae (infective stage).

- **larvae development test:**

100µl of the egg suspension containing approximately (100)eggs was added to each of the six petridishes (90mm in diameter) put in an incubator at (27°C) for 48hours . By then , first stage larvae were developed . The larvae were then exposed to radiation as described above and than returned to the incubator .

Results :

- **Egg viability and hatchability :**

The egg of Haemonchus contortus , Trichostrongylus colubriformis , Oesophagostomum columbianum and Strongyloides papillosus were identified as in method of (Theodoride , 1964) . Microscopic examination of irradiated nematode eggs did not reveal any clear morphological changes with presence of an insignificant number of eggs become darkened in colour in the batches of eggs that exposed to radiation for (60) minutes. As compared to the result obtained in the control , the cumulative percentage (%) hatchability after two days of incubation showed that there was a decrease in hatching rate of eggs that exposed to irradiation (fig.1) The variation in the percentage of egg hatchability compared with the control was not significant at (1) and (5) minutes irradiation level but marked significance appear at (15) , (30) and (60)minutes ($P<0.01$) $d\alpha = 3.08$, 3.25 and 3.76 respectively. No further hatching of eggs occurred in the irradiate eggs after (2) days of incubation.

An additional (2%) of the eggs in the control batch hatched following a further 1-day incubation.As the number of eggs that hatched increased with the duration of hatching , The proportion of hatched eggs at a particular time interval varied with the radiation level (fig.1)

- **Larvae life span :**

As in (table 1) the percentage survival rate reflect that under the experimental condition the control larvae survived for (6) days where as only (19%) of the (60) minutes irradiated larvae survived for (2) days , (100%) of larvae were survive at the control experiment during the same period post-irradiation.

Discussion :

The significant influence of radiation on the living organisms varied and it related to the level of radiation that can disrupt the ability of parasites or other organisms to maintain continuity of their life (Bair and Etges , 1973 ; Anderson et.al , 1982 ; Urbach , 1989). The present study confirms the previous findings. There was a significant interference with the hatching rates of eggs in spite of unobserved drastic changes in the egg morphology or viability of irradiated eggs.

The decreased hatching rate with an increasing UV irradiation exposure is in line with the results of Samuelson et.al (1984). The drop of hatchability in eggs exposed to high level (60)minutes of radiation might have been as a result of radiation damage to the larvae and hence interference with larvae ability to induce hatching (sharma et.al ; 1978) .As reported by Kusel (1970), UV radiation neither can explain any inhibition in the build-up of internal osmotic pressure needed for hatching nor can support the proposal hatching mechanism by Bair and Etges (1973) that hatching may be induced by enzymatic degradation of the egg shell.Higgins-Optiz and Evers (1983) noticed that hatching of eggs occurred as a result of the shell rupturing on one of the two lateral sides of the egg surface. The observed inhibiting effect UV light had on hatchability in this study could have explained such distinctive longitudinal orifice but for localized damage or focal action by enzymes on the rupture line assuming they were present (Samulson et.al , 1984). Radiation could not have selectively affected particular location on the egg shell (Kusel, 1970). On this line of thought , the decreased activity of larvae exposure to high exposure level (60minutes) might have been as a result of radiation damage of the larvae and hence the reduce hatchability rate with increase radiation exposure. The influence of UV radiation on metabolic process of the larvae may directly affect activity and hence survival (Prah and James , 1977) UV radiation also had significant influence on larvae from hatched nematode eggs.Investigation , reveal a reduction in the survival ability of the irradiated larvae , suggesting a possible reduction in transmission with respect to population dynamic of the nematode development . It pertinent to mention that this observation shows a break-point in the lifecycle of irradiated nematode larvae for the purpose of reduction in parasite number and transmission , particularly as there is a level below which on transmission can successfully take place (Krakower , 1994).The present study doesn't establish the direct effect of natural UV radiation from sunlight on the larvae which is positively phototropic. However the increase UV radiation reaching the earth in the future if not controlled , have a significant effect on organisms activity and a reduction in the transmission level of nematode may be occur.

Table 1 : Life span of nematode L1 (larvae) hatched from UV radiated eggs .

Radiation time (mins.)	Time of observation (days)						
	0	1	2	3	4	5	6
0.0	100	100	87	82	80	77	74
1	100	81	68	45	29	2	0
5	100	75	57	25	9	0	0
15	100	69	49	23*	7*	0*	0*
30	100	67	44	19*	5*	0*	0*
60	100	46	19*	0*	0*	0*	0*

* significant difference at level P> 0.01 .

Table 2 : the survival rate of UV radiated nematode larvae

Radiation dose (mins.)	Time of observation (days)					
	1	2	3	4	5	6
0.0	100	100	83	81	77	69
1	100	100	67	31	23	0
5	100	95	51	22	0	0
15	94	66	33*	0*	0*	0*
30	77	47	7*	0*	0*	0*
60	49	19*	0*	0*	0*	0*

* significant difference at level P> 0.01 .

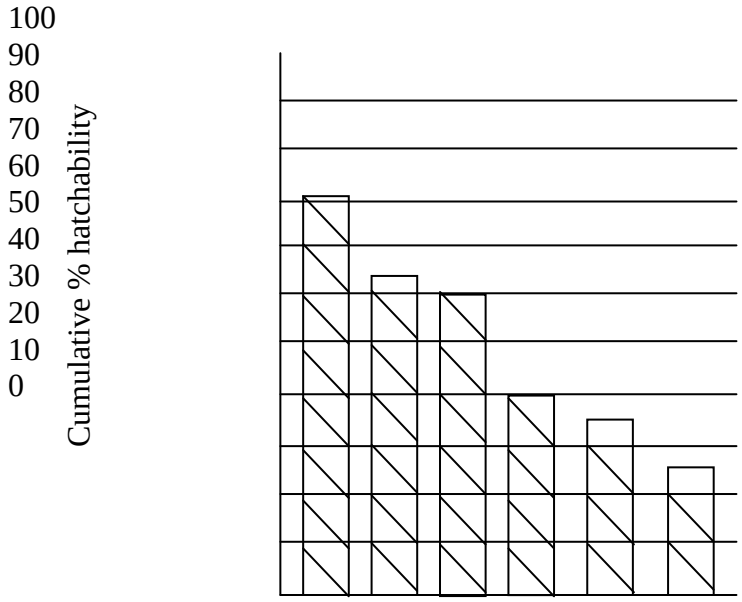

Fig1: cumulative hatchability rate of irradiated nematode eggs

المصادر العربية :

- المحمد نعيم ثاني ؛ الراوي ، خاشع محمود ؛ يونس ، مؤيد احمد والمراني ، وليد خصر (١٩٦٢) مبادئ الإحصاء . مطابع دار الكتب للطباعة . بغداد.

References :

- Anderson , R .M ; Mercer , J.G ; Wilson , R.A. and Carter , N.P.(1982). Transmission of Schistosoma mansoni from man to snail : experimental studies of miracidia and age . Parasitol. 85:339-60.
- Bair , B.D. and Etges , E.J.(1973). Schistosoma mansoni : factors affecting hatching of eggs. Exp. Parasitol. 33:155-67.
-Farvar , M.A. and Cember , H.(1969). Lethal effect of gamma radiation on the schistosomes larvae carrier in snail (Australorbis glabratus). Am.J. Puplic Health .59:2077-80.
-Higgins –Optiz , S.B. and Evers P. (1983). Observations by scanning microscopy of miracidia hatching from Schistosoma mansoni . J. parasitol. 69:43-433.

- Hubert , J . and Kerbouf , D, (1992) . A microlarval development essay for the detection of anthelmintic resistance in sheep nematode.Vet. Rec.130:442-46.
- Keelings , J.E.D. (1960). The effect of ultraviolet radiation on Nippostongylus muris . Irradiation of infective larvae : lethal and sub lethal effect. Ann. Irop .Med .Parasitol . 54:182-91.
- Karkower, C.A.(1994). Some observation on the effect of physical and chemical agents on the cercariae of schistosoma mansoni Puerto Rico . J.public Health Trop. Med . 16:26-44.
-Kusel , J.R. (1970) , studies on the structure and hatching of the eggs of Schistosoma mansoni . J. parasitol. 60: 79-88.
-Litchenberg F, Sadum EW. (1963).Parasitic migration and host reaction in mice exposed to irradiated cercariae of Schistosoma mansoni . Exp. Parasitol.13: 256-65.
- Micheals , R.M. and Kean , B.H.(1969) . X-radiation of S. mansoni In vivo and in vitro effect . AM. J Trop . Med. Hyg. 18:539-44.
- Prah, S.K and James , C. (1977). The influence of physical factors on the survival and infectivity of miracidia of Schistosoma mansoni and S. haematobium .I. Effect of temperature and ultraviolet light . J. Helminthol . 51:73-85.
- Samulson , J.C.; Quinmn , J.J. and Caulfield, J.P. (1984). Hatching chemo kinesis and transformation of miracidia of Schistosoma mansoni J.parasitol. 70:321-31.
- Sharma , V.P.; Razdan , R.K. and Asari , M.A. (1978).Anopheles stephansi ; effect of gamma radiation and chemosterilants on the fertility and fitness of males for sterile male release. J. Econ. Entomol. 71:449-52.
- Stowen , D. (1968). The effect of ultraviolet radiation on Nippostongylus muris . J. parasitol 41:231-34.
- The Nairobi declaration on global climate change . 1990.
-Theodorides ,V.J. (1964) . A simple method for culture and recovery of larvae of intestinal nematodes of sheep .Vet. Rec. 76:353-54 .
- Urbach , F. (1989). Potential effect of altered solar ultraviolet radiation on human skin cancer. Photochem. Photobiol. 50:507-14.
- Weile, W.H. and Mertens , R. (2007). Human wellbeing disease and climate. 2nd edition . Cambridge University press. PP. 345-59.
-Whitlock , H.V.(1948) . Some modification of Macmaster helminthes egg counting technique and apparatus . J. Sci. Ind. Res. Aust. 21:177-80 .