ISOTOPE & RAD. RES., 42(1), 229-238 (2010). *ISSN 0021-1907*
INIS ISRRAC EG

BIOLOGICAL STUDIES ON THE RADIOPROTECTIVE ROLE OF MELATONIN ON THE MEDFLY *CERATITIS CAPITATA* (WIED.), STERILE MALES *

SHOMAN, A.A. and MAHMOUD, E.A.

Biological Applications Department, Nuclear Research Centre,
Atomic Energy Authority, Cairo, Egypt.

Key words: Ceratitis capitata, Gamma radiation, Melatonin, Free radicals, Radioprotection, Male mating competitiveness, Ionizing radiation.

دراسات بيولوجية على دور الميلاتونين الواقى لتأثير الإشعاع على الذكور العقيمة لذبابة فاكهة البحر المتوسط

أحمد عطية شومان و ايمان محمود

خلاصة

تستخدم تقنية الذكور العقيمة بنجاح لمقاومة ذبابة فاكهة البحر المتوسط إلا أن هناك بعض التأثيرات العكسية للأشعة المؤينة التى تستخدم لإحداث العقم على الكفاءة الحيوية لتلك للذكور وهذا بدورة يقلل من كفاءة هذه التقنية. وقد أجريت هذه الدراسة لتقييم التأثير الواقى من الإشعاع للميلاتونين على كل من نسبة فقس البيض و فترة نمو اليرقات و العذارى الناتجة و وزن العذارى ونسبة خروج الحشرات الكاملة و النسبة الجنسية و فترة عمر الذكور و المقدرة على الطيران و الكفاءة التنافسية للذكور العقيمة لذبابة فاكهه البحر المتوسط لتجنب الآثار المؤكسدة الناجمة عن التعرض للإشعاع.

أظهرت النتائج ان استخدام الميلاتونين قبل تطبيق جرعات الإشعاع لم يؤثر على كل من نسبة العقم (نسبة فقس البيض) والفترة اللازمة لنمو اليرقات ووزن العذارى الناتجة بينما أدى إضافة الميلاتونين الى بيئة اليرقات الى زيادة معنوية فى نسبة العذارى الناتجة مقارنة بالمجموعة الضابطة. أشارت النتائج أيضاً الى زيادة معنوية فى كل من نسبة خروج الحشرات الكاملة وفترة عمر الذكور والقدرة على الطيران للذكور الناتجة من عذارى عوملت بالميلاتونين قبل تطبيق جرعات الإشعاع مقارنة بتلك

Accepted March 2010.

التى عولجت بالإشعاع فقط كما أظهرت النتائج أن استخدام جرعات الإشعاع ٩٠ و١٠٠ و ١١٠ جراى بدون المعاملة بالميلاتونين أدى إلى نقص معنو ى فى الكفاءة التنافسية للذكور العقيمة (٠,٣ و ٠,١٧ و ٠,١٩ على الترتيب) كما أشارت النتائج إلى أن إضافة الميلاتونين بنسبة ١ جرام لكل كيلو جرام من بيئة اليرقات ثم تعريض العذارى الناتجة إلى نفس الجرعات السابقة من أشعة جاما أدى إلى تحسن معنو ى فى الكفاءة التنافسية لهذه الذكور (٠,٦٧ و ٠,٤٧ و ٠,٤٦ على الترتيب) عن تلك الذكور التى تعرضت للإشعاع فقط. وقد سجلت النتائج أن اقل كفاءة تنافسية للذكور العقيمة كانت عند تعريض العذارى للجرعة الإشعاعية ١١٠ جراى فقط وان أعلى كفاءة تنافسية كانت للذكور العقيمة التى سبق معاملتها بالميلاتونين قبل تعريض العذارى إلى الجرعة المعقمة ٩٠ جراى، وقد خلصت نتائج هذه الدراسة إلى إمكانية استخدام الميلاتونين كوسيلة سهلة وفعالة لتحسين جودة الذكور العقيمة وزيادة الكفاءة التنافسية للذكور العقيمة التى تطلق لمقاومة ذبابة الفاكهة بتقنية الذكور العقيمة.

ABSTRACT

The sterile insect technique (SIT) has been used successfully against the medfly. The use of gamma irradiation for sterilizing insects has some adverse effects on their competitiveness which in turn reduces the efficiency of the technique. This study was carried out to evaluate the radioprotective effects of melatonin (N-acetyl-5-methoxytyrptamine) on egg hatchability, larval duration, pupal recovery, pupal weight, adult recovery, sex ratio, adult survival, flight ability and mating competitiveness of the medfly, Ceratitis capitata (Wied.), sterile males against the oxidative stress induced by gamma radiation. The results showed that the use of melatonin before the application of radiation doses did not affect the percentages of egg hatchability, larval duration and pupal weight while the addition of melatonin to the larval diet led to a significant increase in the percent of the pupal recovery as compared with the control group. The results also showed significant increases in adult recovery, adult survival and flight ability of males reared on larval diet containing melatonin before irradiation as compared to those treated with radiation alone. The competitiveness values were drastically decreased (0.30, 0.17 and 0.19) with the increase of gamma irradiation (90, 100 and 110 Gy, respectively). However, the results showed that the competitiveness values were increased in males previously reared on a larval diet containing 1 g melatonin/kg larval media (0.1%) and the produced full grown pupae were irradiated with 90, 100 and 110 Gy. The highest male mating competitiveness was recorded when melatonin was added to the larval diet then irradiated the produced pupae with the sterilizing dose (90 Gy). The results

showed that the competitiveness values were 0.67, 0.47 and 0.46 for the doses 90, 100 and 110 Gy, respectively. The results refer to availability of using melatonin as a simple and effective means to improve the quality and the male mating competitiveness of the medfly adult males for release in SIT programs.

INTRODUCTION

The sterile insect technique (SIT) has been used successfully against several insect species (Lindquist et al. 1992, Krafsur, 1998, Tan, 2000 and Koyama et al., 2004). Radiation is used to sterilize medflies, *Ceratitis capitata* (Wied.), for the SIT programmes. Mass-reared flies in the SIT program are sterilized at the pupal stage about 24 hours before adult eclosion with 90-100 Gy using a gamma cell loaded with cobalt-60. However, the use of radiation for sterilizing insects has some adverse effects on their competitiveness which in turn reduces the efficiency of the technique (Cayol et al., 1999, Calkins and Parker, 2005). For successful control with the SIT, it is essential to produce sterile males that will compete successfully with wild males for mating with wild females (Orozco and Lopez, 1993). Radiation interacts with matter by direct or indirect processes to form ion pairs, some of which may be free radicals that lead to molecular damage translated to biochemical damage, may be then amplified and expressed as biological injury (Cockerham et al., 1994). In addition, ionizing radiation affects cells according to their developmental status. Robinson (2002) deduced that the radiation absorbed dose that is used to induce sterility is of prime importance to programs that include the release of sterile insects. Insects that receive too low dose are not sufficiently sterile and those receive too high dose may be uncompetitive, which in turn reduce the effectiveness of the program by requiring great number of sterile insects to be released. Dividing cells are more sensitive to radiation than cells that have reached their terminal stage of development. At full sterilizing radiation doses, non-dividing somatic cells are also damaged and the radiation will decrease the overall quality of the insect, e.g. vigour, longevity and mating competitiveness. As a result, released sterile males typically have relatively low mating success as compared to wild males (Shelly et al., 1994, McInnis et al., 1996 and Lance et al., 2000). Melatonin was identified in vertebrates, invertebrates and insects (Russel et al., 2007). Melatonin was documented as a direct free radical scavenger and an indirect antioxidant. The radioprotective effects of melatonin against cellular damage caused by oxidative stress and its low toxicity make this molecule a potential supplement in the treatment or co-treatment in situations where the effects of ionizing radiation are to be minimized (Haddadi et al., 2006).

In order to reduce the adverse effect of radiation injuries, potent radioprotective substances such as melatonin is applied as a potent scavenger of a variety of free radicals (El-Missiry et al., 2007). The aim of the current study is to investigate the radioprotective effect of melatonin against the oxidative stress and tissue injury induced by the sterile doses of gamma radiation and on egg hatchability, larval

duration, pupal recovery, pupal weight, adult recovery, sex ratio, adult survival, flight ability and the competitiveness of the medfly *Ceratitis capitata* (Wied.) sterile males.

MATERIALS AND METHODS

Rearing and irradiation technique:

The medfly *Ceratitis capitata* was reared in the medfly laboratories of the Egyptian Atomic Energy Authority, Egypt. The rearing technique was carried out as described by Tanaka et al. (1969) at 25°C±2°C and 60-70 % R.H. Adults were fed on sugar and yeast hydrolyzed enzymatically (3:1) (ICN Biomedical Inc.) (Hooper, 1987) and larvae were fed on a standard diet (IAEA, Seibersdorf Laboratory) based on wheat bran and control diet. Full grown pupae were irradiated with 90, 100 and 110 Gy using the ^{60}Co gamma irradiation cell unit installed at the Middle Eastern Regional Radioisotopes Centre for the Arab Countries, Dokki, Egypt.

To investigate the effect of irradiation (90, 100 and 110 Gy) on the efficiency of irradiated males or of irradiated males after rearing on larval diet (containing 1 g melatonin/kg larval media**)** to compete with normal males for mating with normal females, the following mating combinations were undertaken:

- Non-irradiated males (N ♂) : non-irradiated females (N♀).
- Irradiated males (I ♂) : non-irradiated females (N♀).
- Non-irradiated males (N ♂) : Irradiated males (I ♂) : non-irradiated females (N♀).

The competitiveness values and expected egg hatch were computed as described by Fried (1971).

RESULTS AND DISCUSSION

Egg hatchability:

Data in table (1) shows that the percent of hatchability of eggs laid from adults that reared on larval diet containing melatonin (89.29±0.5) was increased significantly ($P<0.05$) than control (80.76±0.7). However, the percentages of egg hatchability laid from fertile females mated with sterile males irradiated only with different applied doses recorded 0.170±012, 0.30±0.02 and 0 %, respectively, which had the same pattern of those treated with melatonin before irradiation (1.22±0.014, 0 and 0.9±0.011, respectively). The data showed that the male sterility was not affected when males were treated with melatonin before irradiation or not.

Larval duration and pupal weight:

Table (1) shows that the larval period (days) and pupal weight were not affected significantly when larvae were reared on normal diet or on diet containing melatonin.

Table (1): Effect of applying melatonin as radioprotective agent and/or different doses of gamma irradiation on percent egg hatchability, larval

duration, pupal recovery, pupal weight and adult recovery of the Mediterranean fruit fly, Ceratitis capitata (Wied.).

Treatment	Egg hatchability %	Larval duration (days)	Pupal recovery %	Pupal weight (mg)	Adult recovery %
Control	80.76 ± 0.71	6.93 ± 0.35	45.47 ± 1.21	0.95 ± 0.014	91.73 ± 2.46
90 Gy	0.17 ± 0.012	6.90 ± 0.21	43.12 ± 2.01	0.95 ± 0.05	86.42 ± 2.29
100 Gy	0.30 ± 0.020	6.95 ± 0.32	44.46 ± 1.86	0.95 ± 0.012	84.61 ± 1.85
110 Gy	0	6.92 ± 0.27	43.34 ± 2.47	0.95 ± 0.08	84.15 ± 2.61
Melatonin	89.29 ± 0.59	6.65 ± 0.47	55.75 ± 1.62	1.02 ± 0.04	98.76 ± 2.05
M + 90Gy	1.22 ± 0.014	6.61 ± 0.26	55.14 ± 1.84	1.02 ± 0.06	95.49 ± 1.47
M + 100Gy	0	6.53 ± 0.42	54.45 ± 2.11	1.00 ± 0.03	95.12 ± 1.61
M + 110Gy	0.9 ± 0.011	6.72 ± 0.31	56.17 ± 2.45	0.99 ± 0.02	94.74 ± 1.52

Pupal recovery:

Percentages of pupal recovery (table 1) from larvae reared on a diet containing melatonin were increased significantly ($P<0.05$) than those reared on normal diet (55.75±1.62 and 45.47±1.21%, respectively).

Adult recovery:

The data in table (1) shows that the percent of adult recovery was significantly increased ($P<0.05$) when applying melatonin to the medfly larval diet (98.76±2.05 %). Moreover, the percentages of adult recovery were significantly increased by values of 95.49±1.47, 95.12±1.61 and 94.74±1.52 when applying gamma irradiation with doses of 90, 100 and 110 Gy, respectively. The adult recovery in the irradiated pupae without melatonin treatment recorded 86.42±2.29, 84.61±1.85 and 84.15±2.61 for the 3 applied doses, respectively.

Sex ratio:

The data in table (2) shows that the sex ratio did not affected neither by applying gamma irradiation to the pupae nor treatment with melatonin during the larval stage.

Table (2): Effect of applying melatonin as radioprotective agent and/or different doses of gamma irradiation on sex ratio, adult survival and flight ability of the Mediterranean fruit fly, Ceratitis capitata (Wied.).

Treatments	Sex ratio (%)		% Adult males survival for 10 days	Flight ability
	♂	♀	♂	
Control	48 ± 0.82	52 ± 0.91	91.29 ± 2.37	88.32 ± 2.64
90 Gy	49 ± 0.59	510 ± 1.02	82.02 ± 3.14	78.64 ± 3.03
100 Gy	51 ± 1.14	49 ± 0.86	79.59 ± 2.63	76.75 ± 2.72
110 Gy	52 ± 1.37	48 ± 1.22	78.00 ± 2.79	76.21 ± 2.43
Melatonin	50 ± 0.93	50 ± 1.08	100.0 ± 0.00	96.54 ± 2.18
M + 90Gy	49 ± 0.87	51 ± 1.33	95.74 ± 2.32	93.11 ± 2.04
M + 100Gy	48 ± 0.79	52 ± 1.61	95.11 ± 2.12	91.32 ± 1.84
M + 110Gy	48 ± 0.83	52 ± 1.81	94.06 ± 1.95	90.02 ± 2.15

Adult survival:

The data in table (2) show that the percent of adult males survival resulted from larval reared on larval diet containing melatonin was increased significantly ($P<0.05$) than control males reared on normal diet (100 and 91.29±2.37 %, respectively). Moreover, the percentages of adult survival were significantly increased (95.74±2.32, 95.11±2.12 and 94.06±1.95) when gamma irradiation (90, 100 and 110Gy, respectively) was applied to the pupae resulting from larvae reared on diet treated with melatonin as compared to those of irradiated pupae without melatonin treatment (82.02±3.14, 79.59±2.63 and 78.00±2.79, respectively).

Flight ability:

Table (2) referred that the flight ability of irradiated males (90, 100 and 110Gy) was decreased with increasing gamma irradiation (78.64±3.03, 76.75±2.72 and 76.21±2.43, respectively) as compared with control (88.32±2.64). However, when melatonin was added to larval diet, significant increase ($P<0.05$) in the flight ability of sterile males (93.11±2.04, 91.32±1.84 and 90.02±2.15, respectively) was observed as compared with those of irradiated without melatonin treatment.

Effect of irradiation only on competitiveness value (CV):

The data in table (3) shows the effect of melatonin and/or gamma irradiation on the male mating competitiveness of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). When larvae were reared on control diet and full grown pupae were irradiated with 90, 100 or 110 Gy, the observed percentages of egg hatchability were significantly increased with increasing gamma irradiation (61.47, 68.55 and 67.42, respectively) as compared with the expected percentages of egg hatchability (40.12, 40.16 and 40.03, respectively).

The male mating competitiveness of irradiated males was decreased when gamma doses increased. At 90 Gy (the sterilizing dose), the male mating CV was 0.3. However, when pupae were irradiated with 100 and 110 Gy, the CV showed more reduction (0.17 and 0.19, respectively). The data shows that exposure of pupal males to gamma irradiation decreased male sterility accompanied by an increase in the observed egg hatchability of irradiated males and a decrease in CV of irradiated males as a side effect of gamma irradiation. The present data agreed with those of Hendrichs et al. (2002) who mentioned that the sterile males lose quality, such that many of them do not compete adequately with wild males for wild females in the field. Moreover, the data corresponded to Cayol (2000) and Lance et al. (2000) stated that the sterile males became less competitive over generation of rearing in the laboratory.

Roushdy et al. (1997) and Abu-Ghadeer et al. (2000) deduced that significant alternations in the physiological and metabolic processes as well as disorders in organ function were observed following gamma irradiation. The negative effects of irradiation on sexual competitiveness of fruit flies are well documented (Holbrook and Fujimoto, 1970, Hooper, 1972, Rossler, 1975, Wong et al., 1983, Kanmiya et al., 1987, Moreno et al., 1991). Lux et al. (2002) described in detail these negative effects on court-ship behaviour of mass reared medflies. In addition, increasing the irradiation dose beyond the 99% egg sterilizing dose greatly reduced the mating competitiveness of males (Fisher, 1997). However, the program managers still demand doses of radiation giving 100% egg sterility resulting in reduced quality of the males for a marginal gain in sterility.

Effect of applying melatonin as radioprotective agent on male mating competitiveness:

The effect of applying melatonin as a radioprotection agent on male mating competitiveness is illustrated in table (3). The data showed that the observed percentage of egg hatchability was significantly increased with increasing gamma irradiation doses (50.82, 57.46 and 57.78, respectively) as compared to the expected percentages of egg hatchability (42.76, 42.10 and 42.60, respectively). The difference between the observed egg hatchability in the irradiated males treated with melatonin was less than the egg hatchability of males that were irradiated only.

The results showed that the competitiveness value of males (which their larvae were reared on diet containing 1 g melatonin/kg media) and full grown pupae (irradiated with 90, 100 and 110 Gy) was increased than males irradiated after treatment with melatonin. The highest male mating competitiveness was recorded when applying melatonin then irradiating the produced pupae with the sterilizing dose (90 Gy). The data demonstrated that the CV was 0.67, 0.47 and 0.46 at the doses 90, 100 and 110 Gy, respectively.

Table (3): Effect of applying melatonin as radioprotective agent and /or different doses of gamma irradiation on the male mating competitiveness value of the Mediterranean fruit fly, Ceratitis capitata (Wied.).

Treatment	Mating combination			Egg hatchability %		Competitiveness value (CV)
	I ♂	N ♂	N ♀	Observed	Expected	
Control	0	1	1	80.07		
90 Gy	1	0	1	0.17		
	1	1	1	61.47	40.12	0.30
100 Gy	1	0	1	0.30		
	1	1	1	68.55	40.16	0.17
110 Gy	1	0	1	0		
	1	1	1	67.42	40.03	0.19
Melatonin	1	0	1	84.29		
Melatonin + 90 Gy	1	0	1	1.22		
	1	1	1	50.82	42.76	0.67
Melatonin + 100 Gy	1	0	1	0		
	1	1	1	57.46	42.10	0.47
Melatonin + 110 Gy	1	0	1	0.90		
	1	1	1	57.78	42.60	0.46

The data obtained confirm the important role of melatonin as radioprotective agent to reduce the bad effect of radiation on the fitness of sterile males. When 90 Gy was applied, the performance of the sterile males was enhanced (0.67) when melatonin was used before applying sterilizing gamma dose as compared to 0.3 in irradiated pupae only. Moreover, the percentage of the observed egg hatchability was 0.17% as compared to 1.22% when melatonin was applied. Furthermore, at the ratio I ♂ : N♂ : N♀, the percentage of egg hatchability without melatonin was 61.47% and reduced to 50.82 % with melatonin. The above mentioned information indicates that the mating ability of sterile males treated with melatonin was improved than those sterile males without melatonin treatment.

In conclusion, the pupal recovery, adult recovery, adult survival, flight ability and mating competitiveness of males medfly reared on larval diet containing melatonin were improved than those irradiated only. The data obtained from the current study showed that melatonin has the potential to overcome most, if not all, of the deleterious effects that occurred due to the utilization of the doses 90,100 and 110 Gy of gamma irradiation. This might indicate its possible use as simple and

effective means in improving the quality and male mating competitiveness of medfly sterile males for the release in SIT programs.

***Acknowledgement*:**

The authors wish to express their gratitude to Prof. Dr. A.M. Wakid, Egyptian Atomic Energy Authority, Egypt, for his help. Thanks are also due to Mr. Sabri Mokhtar and Mr. Waheed Ahmad Sayed for their technical assistances.

REFERENCES

Abu-Ghadeer, A.R., Osman, S.A.A., El-Dardiry, Z.Z., Farag, R.M. and Saad, T.M., Egypt. J. Rad. Sci. Applic., 13(1), 1-15 (2000).

Calkins, C.O. and Parker, A.G., In: Sterile insect quality, Dyck, V.A., Handrichs. J., and Robinson (eds.), Sterile insect technique, Springer, Dordercht, The Netherlands, p. 269-296 (2005).

Cayol, J.P., In: Fruit flies (Tephritidae): Phylogeny and evaluation of behaviour, M. Aluja and A.L. Norrbom (eds.), CRC Press, Boca Raton, FL, USA, p. 84-860 (2000).

Cayol, J.P., Vilardi, J., Rial, E. and Vera, M.T., J. Economic Entomology, 92, 140-145 (1999).

Cockerham, I.G., Mickly, G.A., Walden, T.L. and Stuart, B.O., Press Ltd., New York, Ch. 13, p. 456 (1994).

El-Missiry M.A., Fayed, T.A., El-Sawy, M.R. and El-Sayed A.A., Ecotoxicology and Environmental Safety, 66, 278-286 (2007).

Fisher, K., J. Econ. Entomol., 90, 1609-1614 (1997).

Fried, M., J. Econ. Entomol., 64, 5, 869-872 (1971).

Haddadi, G. and Shirazi, A., IFMBE Proceedings, Imaging the Future Medicine, R. Magjarevic and J.H. Nagel (eds.), COEX Seoul, Korea, Vol. 14, 192, 2194 (2006).

Hendrichs, J., Robinson, A.S., Cayol, J.P. and Enkerlin, W., Florida Entomologist, 85, 1-13 (2002)

Holbrook, F.R. and Fujimoto, M.S., J. Econ. Entomol., 63, 1175-1176 (1970).

Hooper, G.H.S., J. Econ. Entomol., 65, 1-6 (1972).

Hopper, G.H.S., Entomol. Exp. Appl., 44, 161 (1987).

Kanmiya, K.A., Tanaka, H., Kamiwada, K.N. and Nishioka, T., Appl. Ent. Zool., 22, 181-194 (1987).

Koyama, J., Kakinohana, H. and Miyatake, T., Annual Review of Entomology, 49, 331-349 (2004).

Krafsur, E.S., J. Agriculture Entomology, 15, 303-317 (1998).

Lance, D.R., McInnis, D.O., Rendo, P. and Jackson, C.G., Ann. Entomol. Soc. Amer., 93, 1179-1185 (2000).

Lindquist, D.A., Abusowa, M. and Hall, M.J., Medical and Veterinary Entomology, 6, 2-8 (1992)

Lux, S., Vilardi, J.C., Liedo, P., Gaggl, K., Calcagno, G.E., Munyiri, F.N., Vera, M.T. and Manso, F., Florida Entomol., 85, 102-112 (2002).
McInnis, D.O., Lance, D.R. and Jackson, C.G., Ann. Entomol. Soc., 89, 739-744 (1996).
Moreno, O.D.S., Sanches, M., Robacker, D.C. and Worley, J., J. Econ. Entomol., 84, 1227-1234 (1991).
Orozco, D. and Lopez, R.O., In: Fruit flies: Biology and management, Aluja and Liedo (Eds.), Springer-Verlag, New York, p. 185-188 (1993).
Robinson, A.S., Genetica, 116, 5-13 (2002).
Rossler, Y., Entomol. Exp. Appl., 18, 255-257 (1975).
Roushdy, H.M., Abdel-Hameed, F.M. and Abu-Ghadeer, A.R., Isotope and Rad. Res., 29, 11-21 (1997).
Russel, J.R., Dunxian, T., Lucien, C.M., Artemis, P.S., Maria, D.M., Luis, J.F. and Pilar, M.T., World Rev. Nutr. Diet Basel, Karger, 97, 211-230 (2007).
Shelly, T., Whittier, T. and Kaneshiro, K., Ann. Entomol. Soc. Amer., 87, 470-481 (1994).
Tan, H.K., The 5th International Symposium on Fruit Flies of Economic Importance, 28 May-5 June 1998, Penang, Malaysia (2000).
Tanaka, N., Steiner, L.F., Ohinata, K. and Okamoto, R., J. Econo. Entomol., 62, 967 (1969).
Wong, T.Y., Nishimoto, J.I. and Melvin Couey, H., Ann. Entomol. Soc. Amer., 76, 51-55 (1983).