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# Electron Beam Irradiation of Immature Stages and Adult Males of Two Spotted Spider Mite, *Tetranychus urticae* KOCH (Acari : Tetranychidae)

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**Abstract**: Immature stages and adult males of *Tetranychus urticae* were irradiated with electron beams to develop an alternative quarantine treatment for imported cut flowers. Immature stages irradiated at 0.4~0.8 kGy increased tolerance with their development. Females were more tolerant than males during immature stages, but they were completely sterilized even at 0.4 kGy. When irradiated adult males mated with non-irradiated virgin females, there was no significant reduction of mortality. Ovipositional rate (eggs/♀/day) significantly varied and hatchablity of progeny decreased with increasing doses. Males irradiated at 0.2 kGy produced both sexes of progeny. At 0.4 kGy, males were sterilized and females mated with them yielded only male progeny. However, the males recovered their fecundity 10~14 days after irradiation, although their female progeny was malformed and sterilized.

**Key words**: Arachnida, *Tetranychus urticae*, radiation, sterility, immature stage, adult male

## Introduction

Arthropod pests which are found on imported cut flowers are mostly thrips, mites, Lepidoptera and aphids. Their high fecundity and chemical resistance cause difficulty in controlling them in the field and greenhouse (SMITH and FULTON, 1951 ; DENNEHY, *et al.*, 1987 ; GRAFTON-CARDWELL, *et al.*, 1987). Especially, harmful are the invasion of pests which do not occur in Japan resulting in severe economic damage on domestic horticulture. Thus irradiation with gamma rays has been studied in order to develop a new quarantine treatment of cut flowers (WIT and VRIE, 1985).

Lethal and sterilizing effects of electron beam irradiation on eggs and adult females of *Tetranychus urticae* showed possible application to plant quarantine (DOHINO and TANABE, 1993). The sterilizing dose in females was determined using their parthenogenesis, in which virgin females yielded unfertilized eggs fated to be haploid males, while mated females yielded unfertilized and fertilized eggs fated to be diploid females (GUTIERREZ, *et al.*, 1970 ; HELLE and BOLLAND, 1967 ; HELLE, *et al.*, 1970 ; SCHRADER, 1923).

In the present study, the effects of electron beam irradiation on immature stages (larva ~teleiochrysalis) and adult males of *T. urticae* were investigated, and efficacy of irradiation as a quarantine procedure was discussed.

## Materials and methods

1. Rearing

*T. urticae* was obtained from the Faculty of Horticulture, Chiba University. The mite colony was reared with kidney bean leaves at 22°C±1°C and 70% r.h. under a photoperiod of 16L : 8D. Under these conditions, the duration of egg, larva, protochrysalis, protonymph, deutochrysalis, deutonymph and teleiochrysalis was 4～7 days, 1 day, 1～2 days, 1～2 days, 1～2 days, 2 days and 1～2 days, respectively. Adult males and females emerged about 6 and 9 days after hatch, respectively.

2. Immature stages

Five females and 1 male were allowed to oviposit on a bean leaf which was put on agar medium (DOHINO and TANABE, 1993). After 24 hours, the mites were removed, and the number of eggs laid on the leaf was recorded. The immature stages from these eggs were held under the rearing conditions until irradiation. They were divided into two groups and irradiated 6 or 9 days after oviposition. The former was the larval group which was consisted of larva, protochrysalis and protonymph at the ratio of 40.5 : 57.1 : 2.4 (n=225). The latter was the deutonymphal group of protonymph, deutochrysalis, deutonymph and teleiochrysalis at the ratio of 8.3 : 26.0 : 48.7 : 17.0 (n=261). After irradiation, adult emergence and sex ratio of the immature stages, as well as the number and hatchability of their progeny were recorded.

3. Adult males

Adult males emerged from unfertilized eggs yielded by 5-day-old virgin females. Five-day-old males were irradiated and mated with non-irradiated virgin females. A pair of them was reared on a bean leaf and was transferred onto a new leaf every 3 or 4 days. A new female mite was also exchanged every 3 or 4 days. Ovipositional rate, and hatchablity and sex ratio of progeny were recorded.

4. Irradiation

Mites were irradiated with an electron beam accelerator according to the previous study (DOHINO and TANABE, 1993 ; HAYASHI, *et al.*, 1992). After irradiation, mites were held under the rearing conditions.

## Results

Irradiation of the larval group (larva～protonymph) resulted in delayed development and decrease of population, and these effects were intensified with increasing doses. Immature duration of the larval group, which was 12.3 days in non-irradiated control, extended to 18.1 days and 19.5 days at 0.4 kGy and 0.6 kGy, respectively. Adult emergence in the larval group was inhibited at 0.4 kGy and 0.8 kGy for males and females, respectively (Table 1). Adult females from the irradiated larval group did not oviposit.

Adult emergence of males in the deutonymphal group (protonymph～teleiochrysalis)

**Table 1.** Adult emergence, mortality and fecundity of irradiated larval group (larva~protonymph)

| Dose (kGy) | Tested[1] mites | Adult emergence (♀ : ♂)[2] (%) | ♀ Mortality[3] (%) | Eggs laid[4] |
|---|---|---|---|---|
| 0 | 225 | 95.6 (60.0 : 35.6) | 43.7 | 4726 |
| 0.4 | 217 | 36.9 (36.9 : 0 ) | 8.8 | 0 |
| 0.6 | 220 | 7.3 ( 7.3 : 0 ) | 50.0 | 0 |
| 0.8 | 222 | 0 ( – ) | – | – |

1) The amount of 6 replications

2) Adult emergence $=\frac{\text{adults}}{\text{tested mites}}\times 100$ (%)

3) Mortality 2 weeks after irradiation $=\frac{\text{dead adult females}}{\text{adult females}}\times 100$ (%)

4) The amount of eggs which were yielded within 2 weeks after irradiation.

**Table 2.** Adult emergence, mortality and fecundity of deutonymphal group (protonymph~teleiochrysalis)

| Dose (kGy) | Testet[1] mites | Adult emergence (♀ : ♂)[2] (%) | ♀ Mortality[3] (%) | Eggs[4] laid | Hatchability[5] of progeny (%) |
|---|---|---|---|---|---|
| 0 | 261 | 96.9 (63.6 : 33.3) | 79.5 | 4870 | 89.8 |
| 0.4 | 259 | 90.3 (56.0 : 34.3) | 17.9 | 391 | 0 |
| 0.6 | 247 | 55.9 (47.4 : 8.5) | 22.2 | 69 | 0 |
| 0.8 | 255 | 34.5 (34.5 : 0 ) | 80.7 | 0 | – |

1) The amount of 7 replications

2) Adult emergence $=\frac{\text{adults}}{\text{tested mites}}\times 100$ (%)

3) Mortality 2 weeks after irradiation $=\frac{\text{dead adult females}}{\text{adult females}}\times 100$ (%)

4) The amount of eggs which were yielded within 2 weeks after irradiation.

5) Hatch was observed for 10 days after oviposition.

was inhibited at 0.8 kGy (Table 2). The males remained at deutochrysalis and could not emerge, while sterilized adult females emerged at that dose. Although the females oviposited at 0.6 kGy or less, laid eggs did not hatch. Most of these eggs, for example 81.6% of them at 0.4 kGy, was yielded by females irradiated at teleiochrysalis.

In the both immature groups, mortality of females after adult emergence increased with dose. However, maximum mortality, which was obtained at maximum dose, was equal to the mortality of the non-irradiated control. Irradiation at immature stages caused the body color of adult females to change from dark green to light orange or white-yellow.

Irradiation to adult males resulted in a significant increase in the ovipositional rate, and in a remarkable reduction of hatchability of their progeny (Table 3). The ratio of females in adult emergence of progeny decreased with dose, while adult males emerged at a constant rate as same as the non-irradiated control (Fig. 1).

**Table 3.** Fecundity of irradiated adult males

| Dose (kGy) | Days after irradiation | Tested pairs | Ovipositional rate[1] (eggs/♀/day) | Progeny[2] | | Sterility[3] (%) |
|---|---|---|---|---|---|---|
| | | | | Hatchability (%) | Adult emergence (♀ : ♂) (%) | |
| 0 | 0～ 3 | 29 | 6.4±2.2 | 97.3 | 90.8 (58.8 : 32.0) | 3.4 |
| | 3～ 7 | 27 | 4.0±1.8 | 95.8 | 85.2 (60.6 : 24.6) | 3.7 |
| | 7～10 | 21 | 5.5±1.6 | 94.5 | 90.5 (59.9 : 30.6) | 0 |
| | 10～14 | 16 | 4.1±1.5 | 96.5 | 92.6 (61.2 : 31.4) | 6.3 |
| 0.2 | 0～ 3 | 24 | 4.7±1.6** | 52.1 | 30.1 ( 2.4 : 27.7) | 70.8 |
| | 3～ 7 | 20 | 6.8±2.1** | 63.1 | 48.3 (15.2 : 33.1) | 20.0 |
| | 7～10 | 14 | 4.5±1.5 | 55.1 | 23.0 ( 5.4 : 17.6) | 50.0 |
| | 10～14 | 10 | 9.1±1.5** | 56.9 | 44.5 ( 7.7 : 36.8) | 30.0 |
| 0.4 | 0～ 3 | 27 | 4.9±1.9* | 41.3 | 31.3 ( 0 : 31.3) | 100 |
| | 3～ 7 | 23 | 6.4±2.4** | 36.2 | 31.1 ( 0 : 31.1) | 100 |
| | 7～10 | 19 | 6.1±2.0 | 43.3 | 22.9 ( 0 : 22.9) | 100 |
| | 10～14 | 16 | 8.1±1.6** | 40.2 | 35.0 ( 0.4 : 34.6) | 87.5 |

1) Ovipositional rate of non-irradiated females mated with irradiated males.
2) Hatch and adult emergence were observed for 10 days and 2 weeks after oviposition, respectively.
3) Sterility $= \frac{\text{sterilized males}}{\text{tested males}} \times 100$ (%)
*; Significant (t test, $P<0.05$)
**; Significant (t test, $P<0.01$)

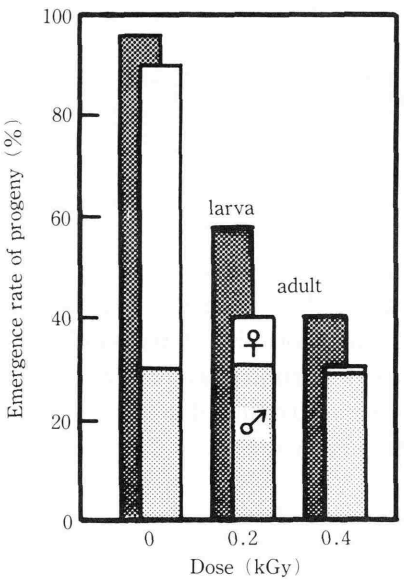


**Fig. 1.** Hatch and adult emergence of progeny yieled by irradiated adult males. Data are based on Table 3.

## Discussion

Females were more tolerant against irradiation than males during immature stages. A similar tendency was reported for eggs of *T. urticae* (DOHINO and TANABE, 1993), and larvae and deutonymphs of *T. arabicus* (ELBADRY, *et al.*, 1972; WAKID, *et al.*, 1972b). Phenotype seems to cause the difference of sex in radiosensitivity of somatic cells in terms of differentiating.

Immature stages of *T. urticae* increased tolerance with their development. In the previous research, eggs also showed similar tendency (DOHINO and TANABE, 1993). Mature eggs were much more tolerant than the larval and the deutonymphal group, comparing the emergence rate into the subsequent stage. $LD_{50}$ for the emergence rate of 5-day-old eggs, the larval and the deutonymphal group to the next stage was 1.3 kGy, 0.3 kGy and 0.7 kGy, respectively. Thus, momentary radiosensitivity of immature stages showed various levels. However, $LD_{50}$ for the adult emergence of 5-day-old eggs, the larval and the deutonymphal group was 0.4 kGy, 0.3 kGy and 0.7 kGy, respectively. It appears that the adult emergence rate of irradiated immature mites increased in stages, since the mortality at given time was repeatedly modified by metamorphoses and was cumulated.

After adult emergence, mortality of females irradiated at lower doses was lower than that of the non-irradiated control (Table 1 and 2). Since the irradiated females were inactive, hardly fed and oviposited, it seems that they spent less energy than the non-irradiated control and represented lower mortality. In higher doses, although similar inactivation occurred, the lethal effect of irradiation probably increased mortality.

When irradiated males mated with non-irradiated females, ovipositional rate showed significant variation every two or three days, until 10 days after irradiation (Table 3). After that, the ovipositional rate of irradiated groups was twice higher than control. The variation was observed in the short term, but it was not significant ($P>0.05$) in the total amount during one week as WIT and VRIE (1985) had reported. Ovipositional behavior of non-irradiated females may be influenced by mating with sterilized males.

The imbalance of the sex ratio in progeny yielded by irradiated males and non-irradiated females fairly shows that the dominant lethal mutation in the irradiated male gamate or sperm resulted in death of fertilized eggs of female progeny (Fig. 1). A similar effect of irradiation was reported in other arthropod organisms which exhibited arrhenotokus reproduction (HENNEBERRY, 1964; WAKID, *et al.*, 1972a; BEAVERS, *et al.*, 1971; HEIDENTHAL, 1945; von BORSTEL, *et al.*, 1955).

Most of *T. urticae* males irradiate at 0.2 kGy recovered their fecundity 3~7 days after irradiation (Table 3). While at 0.4 kGy, two males recovered 10~14 days after irradiation and yielded only two females which were malformed and sterilized. Ultrastructural research for *Acarus siro* males irradiated with electron beams revealed that spermatogonia and spermatozoa were more tolerant than spermatocytes and spermatids, and suggested that primary gonial cells repopulated testes at 0.3 kGy and less (SZLENDAK, *et al.*, 1992). It seems that irradiation at 0.4 kGy prevented the primary gonial cells of *T. urticae* from completely repopulating spermatozoa.

It is concluded from our previous and present study that electron beam irradiation is able to kill or sterilize all stages of *T. urticae* at 0.4 kGy (DOHINO and TANABE, 1993). The dose does not cause severe injuries on many kinds of cut flowers (TANABE and DOHINO, 1993; TANABE and KATO, 1992; WIT and VRIE, 1985). The electron beam irradiation is an effective quarantine treatment to control mites on cut flowers.

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## 和 文 摘 要

# ナミハダニ *Tetranychus urticae* KOCH (Acari : Tetranychidae)の電子線照射

## ——生育ステージおよび雄成虫に与える影響——

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ナミハダニ *Tetranychus urticae* の生育ステージ（幼虫～第3静止期）および雄成虫に2.5 MeV電子線を照射して，その影響を調べた。

幼虫～第3静止期を照射したとき，発育につれて耐性が高くなった。また，すべての生育ステージにおいて，雄よりも雌の方が耐性が高かった。0.4 kGy以上の照射区から得られた雌成虫は完全に不妊化されていた。

雄成虫を照射し，非照射の未交尾雌と交配させたとき，線量増加に伴って次世代の孵化率と雌子孫の割合が低下し，優性致死効果が認められた。