



## Electron-beam irradiation as a quarantine treatment against red mite on exported cut flower of *Chrysanthemum*

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**Abstract:** Utilization of phytosanitary irradiation as a potential treatment to disinfest agricultural commodities in trade has expanded rapidly in the recent years. In this study, red mites (*Tetranychus sp*) isolated from cut *Chrysanthemums* were treated by electron beam irradiation at doses of 100, 200, 300 and 400 Gy. After irradiation, they were kept at room temperature to determine the effects of electron beam irradiation on red mite at different developmental stages (eggs, nymphs and adults) and the reproduction of female adults. The results showed that the pattern of tolerance to irradiation in red mite was eggs < 2<sup>nd</sup> instars < adults, in which the adults were the most tolerance stage. The number of eggs laid by the irradiated female mites and the hatching rate significantly decreased when the irradiation dose increased. The hatching rate of eggs at 300 Gy was 4.25% and no larvae F<sub>1</sub> survived during observation time. It could be concluded that 300 Gy is the effective quarantine dose for red mite infesting on cut *chrysanthemums*.

**Keywords:** *Chrysanthemum*, Electron Beam, Irradiation, Quarantine treatment, Red mite

### I. INTRODUCTION

Vietnam has a tropical monsoon climate. This condition is suitable for many kinds of flowers. The area of cut flower production in Vietnam increased 2.3 times, the output value grew 7.2 times from 2005 to 2015, reached 6.500 billion VND. However, the flower export value is only \$ 60 million out of more than \$ 30 billion for the export of agricultural products, forestry and fisheries. This figure is insignificant compared to the total trade of 130 billion USD of the flower in the world. There are countless varieties of *Chrysanthemums*. Beside traditional yellow, other colours such as white, purple, and red are available. In Vietnam, the planting area of *Chrysanthemum* has increased year by year and now estimated occupying about 30% of the total cut flower area in Vietnam. Export value of

*Chrysanthemum* to Japan is approximately 194 thousands USD per year. However the figure is very limited in the volume compared with Japan's *Chrysanthemum* flower imports. To improve the value export, we need not only new types of flowers and application of advanced cultivation techniques but also quarantine treatments [1].

Cut *Chrysanthemums* are subjected to restrict for plant pest quarantine especially red mites. Therefore, they are treated by using tradition chemical such as methyl bromide or insecticide solution before shipment to international markets. However, methyl bromide will be phased out because it is ozone depleting substance [2] and unsafe for workers and the environment. In addition, the fumigation can not treat a large quantity of flowers simultaneously and takes several hours

to complete the treatment. Therefore, alternative methods to replace methyl bromide fumigation are required. The method that can treat a large number of flowers is irradiation treatment [3]. There are three primary types of irradiation capable of phytosanitary treatment such as gamma, electron beam and X-ray. In this study, electron beam were used to irradiate insect pests infesting cut flowers. Despite reduced penetration, electron beam processing allows for much faster and more precise processing than other treatment options [4]. Additionally, the efficiency of electron beam processing often means that no warehousing of product is necessary before treatment a significant advantage for cut flowers. Moreover, the most significant advantages of electron beam processing are that the technology does not require the use of radioactive isotopes. This significantly reduces a number of security concerns, simplifies permitting and regulatory requirements, and eliminates the costly and complicated processes of acquiring, transporting, installing, and eventually replacing radioactive sources [5]. At the doses required for phytosanitary treatments, electricity based processes such as electron beam are also more cost-effective than cobalt-60 [6].

## II. EXPERIMENTAL

### Materials

Red mites, thrips and leafminers were investigated on *Chrysanthemums* in ward 8, Da Lat city, Lam Dong province to assess the level of their damage. Red mites the most infected pests were collected, transferred to the biology laboratory of Research and Development Center for Radiation Technology and reared on leaves of *Chrysanthemums* (28 ± 2°C temperature, relative humidity of 80 ± 5%).

### Direct investigation of the main harmful insects on cut flowers

*Chrysanthemums* which were blooming on one farm at 5 points diagonally were

collected and observed by stereomicroscope. Ten consecutive plants at the point and two leaves for one plant were investigated the insects such as red mites, thrips and leafminers. After that, the plants were put in separate plastic bags and transferred to the laboratory. Time for investigation was during January. Calculating the population density of insect by the formula:

Density (insect/leaf) = total investigated insect/total investigated leaf.

Assessing insects infesting in flowers was expressed by symbols as following:

- No appearance
- + There is damage but no significant damage (< 1 insect/leaf or 1 insect/long stem (5-7 cm))
- ++ There is average appearance and average damage (1-4 insects/leaf) or long stem (5-7 cm)
- +++ There is common appearance, significant and heavy damage (over 4 head/ leaf) or long stem (5-7 cm).

### Irradiation treatment

The red mites were isolated from most infected leaves, reared on the same object and their developmental stages were observed at laboratory conditions. Different developmental stages of red mite (eggs, 1<sup>st</sup> instars, 2<sup>nd</sup> instars, 3<sup>rd</sup> instars, and adult) on detached leaf were irradiated with doses ranging from 0-400 Gy by electron beam accelerator UERL-10-15S2, 10 MeV at Research and Development Center for Radiation Technology with parameter  $F_{syn} = 70$  Hz,  $v = 4.2$  m/min. Fricke dosimeter were used to measure absorbed dose. After irradiation, the samples were held at standard rearing condition (28-31°C, 80-85% RH) to observe mortality rate, survival rate of nymphs and female adult reproduction.

### Data analysis

Experimental data were analyzed of variance (ANOVA) using Statgraphics 15.0 with the reliability  $P = 0.05$ .

### III. RESULTS AND DISCUSSION

#### Direct investigation of the main harmful insects on cut flowers

The result showed that red mites were common, causing significant damage on *Chrysanthemum* (Table I). This kind of pest focused on midribs and suck tissues of leaves

and flowers. If the leaves and flowers infested red mites seriously, they would have brown color and fall down. Under the above rearing conditions, the total life cycle of red mite was 15 days. The common appearance stages on cut *Chrysanthemum* were eggs, 2<sup>nd</sup> instars and adults. In this study, red mites were chosen to be controlled by irradiation.

**Table I.** The pests infesting on *Chrysanthemum* cultivated in ward 8, Da Lat city, Lam Dong province

Name flowers	Name pests	Level of damage	The most infected stage of the life cycle
<i>Chrysanthemum</i>	Red mites	+++	Eggs, 2 <sup>nd</sup> instars and adults
	thrips	++	Adults and nymphs
	leafminers	++	Nymphs

#### Effect of irradiation on development of eggs to adults

The data indicated a positive relationship between radiation dose and egg mortality. Hatching rate of eggs decreased with the increasing absorbed dose (Table II). Mortality of 1-2-day-old eggs of the red mite were 10.56; 94.59; 97.14; 100 and 100% after treatment with doses of 0; 100; 200; 300, and 400 Gy, respectively. At low doses less than 300 Gy, the number of eggs still hatched, but effects of

irradiation were reflected by mortality in subsequent stages. No adults of the red mite developed from 1-2-day-old eggs exposed to doses more than 100 Gy. The increase in mortality of immature stages developing from irradiated eggs was usually observed in red mites. Similar results were also reported by Hu et al, 2004 [7]; Ignatowicz and Banasik-Sol gala, 1999 [8].

**Table II.** The development of irradiated eggs of red mites

Dose (Gray)	Number of eggs	1 <sup>st</sup> instars (%)	2 <sup>nd</sup> instars (%)	3 <sup>rd</sup> instars (%)	Adults (%)
0	170	89.44 ± 2.35 <sup>a</sup>	84.09 ± 3.05 <sup>a</sup>	81.09 ± 2.75 <sup>a</sup>	79.77 ± 0.00 <sup>a</sup>
100	150	5.41 ± 2.00 <sup>b</sup>	0.78 ± 1.35 <sup>b</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>
200	140	2.86 ± 2.15 <sup>bc</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>
300	165	0 ± 0.00 <sup>c</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>
400	137	0 ± 0.00 <sup>c</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>	0 ± 0.00 <sup>b</sup>

Mean values within the same stage followed by the same letter are not significant different at  $P=0.05$ .

#### Effect of irradiation on development of 2<sup>nd</sup> instars to adults

The obtained results in Table III indicated the effects of irradiation on survival of 2<sup>nd</sup> instars at subsequent developmental stages. Mortality of red mites that developed from

irradiated 2<sup>nd</sup> instars increased with the increase of radiation dose. The percentage of adults that developed from non-irradiated 2<sup>nd</sup> instars was 94.34%. Meanwhile, these rates were 78.13, 57.95, 0.92 and 0% in adults that developed from 2<sup>nd</sup> instars treated with doses of 100, 200,

300, and 400 Gy, respectively. We did not observe any female adult emergence from irradiated 2<sup>nd</sup> instars at dose 300 Gy. It could be concluded that the dose of 300 Gy might be efficient to sterilize the red mites developed from irradiated 2<sup>nd</sup> instars. Similar result was also observed by Ignatowicz and Banasik-Solgala, 1999. However, Hu et al, 2004 reported that the mortality of 2<sup>nd</sup> instars was 100% at dose 100 Gy after 10 days of irradiation. This might be because the temperature for rearing were quite different in each experiments.

### Effect of irradiation on survival and reproduction of female adult

The mortality and the number of eggs of irradiated female adults showed in Table IV. The mortality rate of female increased after 15 days and reached to 100% at dose 300, 400 Gy. Viability of eggs laid by irradiated females decreased with the increase of dose. Hatching rate of eggs produced by treated red mite adults at dose 300 Gy was 4.25%. However, the number 1<sup>st</sup> instars of F<sub>1</sub> generation didn't develop to 2<sup>nd</sup> instars during the observed time. It can be estimated that 300 Gy was dose required to sterilize red mites by electron beam irradiation.

**Table IV.** Effect of irradiation on survival and reproduction of female adult

Dose (Gray)	Number of red mites adults	Mortality rate (%)	Number of eggs/ /female adults	Hatching rate of eggs (%)
0	58	16.49 ± 4.31 <sup>a</sup>	2759 ± 23 <sup>a</sup>	93.6 ± 1.46 <sup>a</sup>
100	45	55.33 ± 7.92 <sup>b</sup>	256 ± 12.53 <sup>b</sup>	63.68 ± 5.78 <sup>b</sup>
200	47	77.71 ± 7.45 <sup>c</sup>	187 ± 10.15 <sup>c</sup>	41.5 ± 2.08 <sup>c</sup>
300	53	100 ± 0.00 <sup>d</sup>	81.67 ± 9.02 <sup>d</sup>	4.25 ± 2.91 <sup>d</sup>
400	57	100 ± 0.00 <sup>d</sup>	43.33 ± 4.73 <sup>e</sup>	0 ± 0.00 <sup>d</sup>

Mean values within same stage followed by the same letter are not significant different at  $P=0.05$ .

## IV. CONCLUSIONS

All electron beam irradiation doses used in this study impeded the development of immature stages of red mites and caused eventual death in comparison with the control. The survived adults after radiation were not able to create new generations. The insect immature stages were more sensitive to irradiation than the adult ones. The pattern of tolerance to irradiation in red mites was eggs < 2<sup>nd</sup> instars < adults. The mortality rate of red mite adults achieved 100% at dose  $\geq$  300 Gy after 15 days. The number of eggs laid by irradiated females was considerably lower than in the control, and decreasing with increasing radiation dose. The 2<sup>nd</sup> instars of F<sub>1</sub> generation were not observed in the treatment involving

irradiation of adults at 300 Gy. The dose of 300 Gy might be efficient dose to sterilize red mites infected on *Chrysanthemum*. However, the effect of irradiation on red mite needed implementation on large scale confirmatory tests and the quality of exported flowers after irradiation should be evaluated.

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