

The Effect of Gamma Radiation Induced Sterility in Potato Tuber Moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)

M. A. Naji¹, A. A. Kafu², N. A. Mahfoud¹, and H. A. Maghrabi¹

¹Zoology Dept. Faculty of Science, University of Tripoli

²Biotechnology Research Center, Tripoli, Libya

Abstract

A total of 400 pupae/cage were transferred into sterilized cages for laboratory rearing of potato tuber moth, PTM, *Phthorimaea operculella* Zeller at the Agriculture Research Center, Tripoli. Emerged adults in each cage fed 10% sugar solution on soaked wicks. After mating and oviposition eggs were collected daily. A batch of cleaned and sterilized potatoes was punctured and tubers infested with PTM white eggs. After hatching larvae find their way into tuber through entrance holes. Infested potatoes were placed in rearing trays to pupate in the sand. Pupae were collected, sieved and removed into sterile rearing cages for adults emergence. Adult male parents 24h old were radiated with gamma radiation 100, 150 and 200 Gy at the Nuclear Research Center, Tajura. Adult mating crosses including control were evaluated for the effects of induced gamma radiation sterility in PTM. Significant differences of gamma radiation effects to PTM male parents were observed for reduction counts of eggs, pupae and adult males at 0.05 level and females at 0.001 level for 150 and 200 Gy gamma radiation doses. This study indicates that the use of sub-sterilizing doses of gamma radiation could be considered as an important component in the IPM control strategy.

Keywords: *Phthorimaea operculella*; Gamma radiation; Botatote Moth; Induced sterility; Lepidoptera.

المستخلص

تم تربية مجموع 400 عذراء معقمة لكل صندوق لتربية فراشة درنات البطاطس *Phthorimaea operculella* Zeller وتغذية الحشرات البالغة بمحلول سكري 10% معمليا بمركز البحوث الزراعية، طرابلس. عند اكتمال عملية التزاوج جمع البيض يوميا ووضع على دفعات فوق درنات البطاطس النظيفة المثقبة يدويا لغرض الإصابة. وبعد فقس البيض تمر اليرقات الصغيرة خلال الدرنات للغذاء، وتوضع الدرنات المصابة في صينية التربية مزودة برمل معقم للتعذر. جمعت العذارى في أقفاص التربية للحصول على البالغات لفراشة درنات البطاطس. الذكور الآباء لفراشة درنات البطاطس بعمر 24 ساعة تم معاملتهم بأشعة جاما بجرعات 100، 150، 200 جراي بمركز الأبحاث النووية، تاجوراء. تم تقييم تهجين البالغات لفراشة درنات البطاطس مع الشاهد لأثر عملية العقم بأشعة

جاما. أثر أشعة جاما الموروث للذكور بات واضحاً وبفروق معنوية لمستوى 0.05 لخفض الكثافة العددية للبيض والعداري والحشرات الكاملة، خاصة عند الجرعة 200، 150 جاري، وبفروق معنوية عالية لمستوى 0.001. تؤكد هذه الدراسة على الأهمية البالغة لأثر استخدام تشعيع ذكور فراشة البطاطس *P. operculella* الجزئي ضمن إستراتيجيات وأساليب مكافحة الكاملة للآفات.

Introduction

The potato tuber moth (PTM) *Phthorimaea operculella* (Zeller), (Lepidoptera: Gelichiidae), is the most damaging pest of potatoes; *Solanum tuberosum* L., Solanaceae, in warm temperate and subtropical climates (Fenemore, 1988). During the growing season, the potato tuber moth mines into foliage and stems, while at plant senescence adults lay eggs in soil and exposed tubers. At harvest, tubers do not always show signs of damage but may harbor eggs and early instar larvae. This may result in severe losses in the absence of adequate control measures (Ahmed et al, 2013).

The PTM is the single most significant insect pest of potatoes in North Africa and Middle East (Fuglie et al., 1992). In Libya farmers routinely apply chemical insecticides to protect their crops. This reliance on chemical insecticides not only increase costs but also has serious deleterious effects on human health, environment and development of insect resistance to insecticides. The annual potatoes production in Libya is more than 100.000 tons. Potatoes are grown in two seasons the spring and the fall crops, mainly on the coastal areas. Potatoe varieties for Libyan market are mainly imported from Europe and Canada. The domestic availability of potatoes in Libya averages the amount of 129.000 tons, 83% is used for human consumption, 12% as seed, and 5% is wasted (CIP, 1974; Fhema, 1976).

PTM has a complete metamorphosis. The insect is present over winter as larvae or pupae in the soil or in potatoes tubers. Most active at dusk and dawn, the weak-flying moths emerge in spring and flutter from plant to plant. Each female deposits 60 to 200 eggs in 4 days or less. Eggs usually are placed on rough surface such as hairy underside of a leaf. Hatch occurs 3 to 6 days later, depending on temperature. Larvae feed and mature in 7 to 10 days under ideal summer conditions, but take longer at cooler temperatures. When fully grown, larvae leave their hosts and pupate in the soil near the base of plants, in leaf remains, or in some other suitably sheltered site. A new generation of moths emerges in 6 to 9 days. Five or six generations occur each year (Golizadeh and Esmaeili, 2012).

A substantial body of literature on the potato tuber moth has been developed to understand the biological, physiological and ecological aspects of the destructive insect of potato worldwide (Makee and Saour, 2001). The inherited sterility has been proposed as an alternative control method to this pest. Although the technique requires only sterile male moth to be released, the lack of effective mechanical or and genetic sexing systems for Lepidoptera imposed that both gamma-irradiated males (partially sterile) and females (completely sterile) have to be released (LaChance, 1985). As a result, in the field under sterile insect release program, potentially millions of sterile females could be mating with sterile and possibly fertile males. Depending on the

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applied irradiation dose, an important part of these crosses produce non-viable eggs (Saour and Makee, 1997).

An effective control program may consist of many strategies. Knipling (1970, 1992) and Carpenter (1993, 2000) predicted by mathematical models that the full potential forcing inherited sterility to control (or eradicate) lepidopteran pest may be realized only when inherited sterility is combined with other suppression methods to provide a synergistic pest suppression that is more effective than either technique employed separately.

For control of PTM the combined release of sterile insects and *Trichogramma* oophagous parasitoids would have the potential of promising control strategy. The inherited sterility will reduce the natural population (sterile F1 adults) and produce non-viable eggs, which can serve as a host material to increase *Trichogramma* and allow them to parasitize egg laid by remaining wild moths (Mannion et al., 1995).

Excessive damage of PTM is caused particularly in warmer regions to stored potatoes. Literature indicates that the potatoes in seed storage were damaged by potato tuber moth in warehouses where potato tubers were stored all year; PTM damage was high 30 – 70 % (Soeriaatmadja, 1990; Roux et al., 1992; Chouvalitwongporn, 1993). Storage of produced potato seed in Libya for the fall planting is a major problem of potato tuber moth infestation (CIP, 1974; Fhema, 1976).

The current status of PTM biology and IPM in Libya are dealt with only few studies. Therefore, the aim of this study is to induce sterility into F1 and F2 generations of potato tuber moth *P.operculella* through gamma radiation of the parent generations with substerilizing doses.

Materials and Methods

Laboratory Rearing of Potato Tuber Moth

Pupae from stock culture at Agriculture Research Center, Sidi Almasry, Tripoli were collected, screened and counted. A total of 400 pupae/cage were transferred into sterilized cages. The newly emerged adults (5 to 7 days) in each cage were fed 10 % sugar solution on soaked wicks. After mating and oviposition white eggs glued on bands of black cardboard were added inside each cage and collected daily.

A batch of 5 kg potatoes were cleaned, sterilized and punctured on the surface with a pin brush to facilitate larval first instars entrance within tubers. Tubers infested with eggs on black cardboard bands. Eggs hatch within 7 to 9 days and newly larvae find their way into tuber through entrance holes. Larval groups wondering around cages were trapped by means of yellow small containers for re-infestation.

After larval metamorphosis and development inside tubers within a period of 9 days, infested potatoes were placed in rearing trays containing sterilized sand layer. Larval fourth instars (16 to 24 days) migrate downwards to pupate in the sand. Pupae were collected, sieved and removed into sterile rearing cages for newly adult emergence (6 to 9 days). After mating and oviposition white eggs are collected for new retrials.

Potato Tuber Moth Induced Adult Radiation for F1 and F2 Generations

Breeding of the F1 Progeny of the Potato Tuber Moth

Newly emerged adult moths (75 male and 75 females) were separated to 5 groups 5 x 15 males and 5 x 15 females. Similarly, including control replicates. Each group put in a cubical cage to induce the required radiation doses at the Nuclear Research Station, Tajura. The adult male moths were radiated at doses: 100, 150, and 200 Gy for 3 hrs. The radiated male moth samples were labelled and immediately carried to the laboratory for further observations.

Radiated males and non-radiated females, 75 each, were singly paired in 350 ml transparent plastic boxes provided with oviposition support and source of food (10% glucose) at required rearing condition. A total of 60 rearing boxes and 60 rearing cages; and 15 replicates for each dose and the control treatment were applied. Females and males were kept together until death. Eggs were removed daily, counted and left until used for reproduction of the F1 progeny. Obtained data were evaluated and statistically analyzed.

Breeding of the F2 Progeny of the Potato Tuber Moth

Newly emerged groups of the F1 progeny are comprised of 5M, 5F, 5M + 5F for each induced irradiated dose, 100, 150, and 200 Gy respectively. Each labeled paired group (1M and 1F) were singly transferred in 350 ml transparent plastic box provided with an oviposition support and 10% glucose solution at rearing requirements.

A total of 370 pairs were evaluated for the following probabilities crosses at each dose:

(Radiated M x non-Radiated F);

(Radiated F x no-Radiated M);

(Non-Radiated M x non-Radiated F) as control treatment.

Females and males were kept together until death. Eggs were removed on a second day bases, counted and left until used for reproduction of the F2 progeny. Adults were counted and evaluated for ANOVA and LSD statistical analyses.

Results

Radiation induced F1 and F2 sterility in PTM, *Pthorimaea operculella*

The total estimate of eggs, pupae and adult males and females of gamma radiated male parents at given different doses were 100, 150 and 200 Gy for F1 and F2 progeny of PTM. In comparison with control treatment and among all applied gamma radiated doses for F1 and F2 generations of potato tuber moth, it was found that 200 Gy dose proved the most effective dose on the reduction and development of F1 and F2 progeny (Table.1).

Eggs.

ANOVA test for the mean effects of gamma radiated induced doses for male parents of PTM and the mean effects of the applied doses through the F1 and F2 generations on eggs counts illustrated that: 1. The mean effects of F1 (66.75) and F2 (49.43) were

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significantly different at 0.05 level ($F = 4.07$; df 1, 352; $p < 0.05$). The effects of induced gamma radiated doses on mean eggs density of F1 and F2 were also significant at 0.05 level, 100 Gy (75.04); 150 Gy (56.69); 200 Gy (38.30), when compared with control treatment (62.34) ($F = 3.2$; 352; $p < 0.05$).

Table. 1. The mean effects of radiation doses on F1, F2 generations, eggs, pupae, adults, females and males PTM counts.

Generation	Radiation Dose	No. Eggs	No. Pupae	No. Adults	No. Females	No. Males
F1	0	70.37	35.33	23.40	11.87	11.53
	100	81.27	26.80	15.13	7.13	8.00
	150	65.80	15.53	10.07	5.53	6.53
	200	49.20	9.07	5.40	2.33	3.06
F2	0	53.95	48.61	23.35	11.83	11.52
	100	68.81	27.37	15.21	7.09	8.12
	150	47.57	16.16	10.57	4.08	6.54
	200	27.40	8.03	4.71	2.03	2.68

The interaction between the generation (F1 and F2) and gamma radiated induced doses for male parents of potato tuber moth indicated that there was no significant difference at 0.05 statistical level among control and introduced radiation doses 100, 150, and 200 Gy. The egg counts were significantly less than the control within each generation and among PTM generations (3,352) ($F=0.05$; $p < 0.9849$).

LSD test revealed that no significant differences between control treatment and 100, 150 Gy doses even though numerical differences were observed. Significant differences at 0.05 level were obtained at 150 Gy (0.0431); 200 (0.00820) doses (Tabl.2).

Table. 2. The LSD test for the main effects of radiation doses of PTM F1 and F2 generations on eggs, pupae, adults, females and males counts.

Radiation Dose	No. Eggs	No. Pupae	No. Adults	No. Females	No. Males
0	.3462	41.97	11.53	11.85	23.87
100	75.04	27.08	8.06*	7.11*	15.17*
150	56.69*	15.85*	6.54**	3.78**	10.32**
200	38.30*	8.55*	2.87**	2.18**	5.05**

*Significant differences at 0,05; **Significant differences at 0.001.

Pupae

The main effects of F1 and F2 progeny on pupal counts results from extended gamma radiated male parents of PTM. It was observed that even though numerical

differences were recorded among F1 and F2 generations, but they were not significant as demonstrated by the ANOVA test.

The mean effects of different gamma radiated induced doses of PTM male parent on pupal development and density were clearly observed as an increase in the applied gamma irradiated dose will result to noticeable decrease in PTM pupal counts, i.e., control (41.97); 100 Gy (27.08); 150 Gy (15.85); 200 Gy (8.55). The ANOVA test showed that insignificant statistical differences were recorded at 0.05 level between induced gamma radiated doses for pupal counts, but significant differences were obtained at 0.06 level ($F = 2.51$; 352; $p < 0.06$), (3,352) ($F=2.51$; $p < 0.0582$).

There was an interaction between the effects of Generation x Doses and slight increase in PTM pupae numbers. ANOVA test outlined that insignificant differences between the interaction effects of Generation x Doses even with clear numerical differences for the effects of induced gamma radiated doses to male parents of PTM (3,352) , $F=.13$; $p < 0.9413$). But, LSD test revealed that significant differences 0.05 level were observed among doses 150Gy (0.0072), 250Gy (0.0061) (Table 2).

Adults

The results demonstrated that the mean effects of radiated gamma induced doses for male parent PTM on adults of the F1 (13.50) and F2 (13.46) progeny were insignificant. Similarly, ANOVA test also revealed that there were no significant differences were observed on effects of applied radiated gamma doses on adults of potato tuber moth.

The increase in gamma irradiated doses on male parent did show a reduction in adult numbers as induced radiation doses increase, i.e., control treatment (23.37); 100 Gy (15.17); 150 Gy (10.32); 200 Gy (5.05). These results were proved by ANOVA test to be highly significant at 0.001 level ($F = 7.47$; 352; $p < 0.001$).

The mean effects of interaction between Generation x Doses on PTM adults for F1 and F2 generations were closely similar .On the other hand, ANOVA test showed that there were no effects due to interaction between generations and doses on potato tuber moth adults(3,352 $F= 0.01$; $p < 0.9991$).

Comparison among mean effects paired gamma radiated induced doses to male parent of PTM, LSD indicated that significant differences were recorded between control treatment and 100 Gy dose at 0.05 level; control treatment and 150, 200 Gy doses at 0.01 levels. A significant difference was revealed between 100 Gy (0.043679), 150 Gy (0.03819) and 200 Gy (0.00001) doses at 0.01 level (Table 2).

Females of PTM

Data showed that mean effects of PTM F1 and F2 progeny through extended gamma radiated male parents on female counts were almost similar. ANOVA test provided that no significant differences for the effects of the first and second generation on PTM female numbers were recorded (1,352) $F =0.00$; $p<.9839$).

Moreover, clear differences were observed on the effects of gamma radiation doses of PTM male parents on produced female counts, i.e., control treatment (11.85); 100 Gy (7.11); 150 Gy (3.78); 200 Gy (2.18). Therefore, higher significant differences at 0.01 level were revealed when higher radiation doses applied for PTM male parents and

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their effects on female numbers as presented by ANOVA test ($F = 10.40$; 352; $p < 0.001$).

ANOVA test also demonstrated that there was an interaction between PTM adult female counts and radiation doses.

The mean effects of interaction between Generation x Doses on PTM adults for F1 and F2 generations were closely similar. On the other hand, ANOVA test showed that there were no effects due to interaction between generations and doses on potato tuber moth female adults.

Similarly, LSD test for pair-wise comparison radiation doses showed that higher significant differences were recorded between control treatment and 100, 150, 200 Gy. But, insignificant differences were obtained between 150 (0.01747) and 200 (0.00046) Gy radiation dose (Table 2).

Males of PTM

The results demonstrated that the effects of induced radiated gamma doses for male parents in both F1 and F2 progeny on males of potato tuber moth were slightly different ANOVA test clarified that insignificant differences were observed on PTM male individuals for F1 and F2 generations (1,352) $F=0.00$; $p < 0.9673$).

Data of this study showed that the mean effects of applied radiation doses for male parent potato tuber moth showed great affect on reduction of PTM male counts, i.e., control treatment (11.53); 100 Gy (8.0); 150 Gy (6.54); 200 Gy (2.87). In contrast, ANOVA test showed significantly higher differences at 0.01 level of induced gamma radiation effects on PTM males ($F = 4.88$; 352; $p < 0.001$).

The mean effects of interaction between Generation x Doses on PTM adults for F1 and F2 generations were closely similar. On the other hand, ANOVA test showed that there was no effects due to interaction between generations and doses on potato tuber moth male adults (3,352) $F=0.00$; $p < 0.9996$).

Nonetheless, LSD test revealed that significant higher differences resulted between control treatment and 100 Gy (0.06631), 150 Gy (0.00018) and 200 Gy (0.00000) (Table 2).

Discussions

Inherited effects in F1 and F2 progeny of gamma radiated males PTM.

Potato tuber moth *P. operculella*, is the most destructive insect pest of potato crops worldwide. In Libya almost 100% losses were recorded. Insecticides extensive use and resistance development to PTM led to considering application of Sterile Male Technique SMT against lepidopteran pests. For example, the inherited sterility phenomenon has been observed in *Laspeyresia pomonella* (Anisimov et al., 1989); *Agrotis ipsilon* (El-Naggar et al., 1984); *Plodia interpunctella* (Brower, 1981); *Spodoptera frugiperda* (Carpenter et al., 1986), and *Helicoverpa zea* (Carpenter et al., 1987). The potential of applying inherited sterility in insect control was demonstrated by Knippling (1970) through a theoretical population control model. The genetic basis of

this type of sterility in Lepidoptera has been reviewed by LaChance (1974 and 1985) and LaChance et al. (1975).

Total of gamma radiation effects on PTM eggs, pupae and adults of PTM

Data of this research contributed essential information about the effects of applying gamma radiation doses (100, 150, and 200 Gy) on male parents PTM and their F1 and F2 progeny. LaChance (1985) reported that all the special characteristics of the F1 progeny related directly to the effect of gamma irradiation on the chromosomal structure of irradiated parents. In Lepidoptera the females are heterogametic "ZW", whereas the males are homogametic "ZZ" (Mittwoch, 1967). The death of the F1 females is caused by the induction of recessive lethal mutations in Z-chromosome of irradiated male parents (Marec, 1990).

In lepidopterous species, the radiation of males with substerilizing doses is associated with high levels of sterility in their F1, F2, and even F3 progeny. LaChance et al. (1973) reported that in general, the use of substerilizing doses of irradiation advised to achieve an effective combination of induced partial sterility and inherited chromosomal aberrations to suppress natural populations. Inherited sterility in F1 and F2 generations of irradiated males of *Spodoptera littoralis*, *Agrotis ipsilon* and *Heliothis zea* were recorded by Wakid and Hayo (1974); El-Naggar et al. (1984); Carpenter et al. (1987).

Total population estimates of gamma irradiation effected PTM males that were exposed to 100, 150, and 200 Gy doses for F1 and F2 generations were observed in both PTM F1 and F2 progeny developmental stages (Tabl.1).

Gamma radiation effects on PTM eggs

Radiation of eggs and pupae of the PTM in comparison to sterile adult male was not feasible. Adult irradiation which was found most ideal for sterility (96.3 %) was induced when emerging males were irradiated. This study is in comparable and agreement with most published data concerning the application of gamma radiation methodology as an effective program in the IPM of PTM.

Data revealed the significance of gamma radiation doses effect (100, 150, 200 Gy) respectively on the production of PTM eggs. The results indicate that at higher radiation doses, 150 and 200 Gy, the mean effects were 65.8 and 49.2 respectively in comparison to control treatment 70.7 for the F1 generation. In contrast, F2 progeny the mean irradiation effects doses were 47.6 and 27.4 compared to control treatment 53.9. But, for both F1 and F2 combined the mean radiation effects were 56.7 and 38.3 respectively as the control treatment was 62.3. The hatchability of eggs was reduced by increasing radiation dose to male parent which was more pronounced in the F2 generation. Doses of 200 Gy produced significantly lower rates of egg hatching than the control. ANOVA and the LSD tests significantly revealed the differences at the 0.01 level (Table 2).

Results indicate that the detrimental effects of radiation continue in the population through F1 and to F2. Among F2, significant reduction in egg hatch indicated that deleterious factors were still present in the population. PTM egg mortality is considered to be of great value in the reduction of PTM population in IPM strategy and control.

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Larval mortality due to radiation application doses to PTM male parents for F1 and F2 progeny were mainly observed through egg hatchability.

Gamma radiation effects on PTM pupae

Adult male parents PTM radiation showed higher incidence of adult deformity and losses at higher doses of 150 and 200 Gy due to the effects on pupal developmental stages with mean effects 15.5 and 9.1 respectively as compared to control treatment 35.3 for F1 generation. In contrast, the pupal F2 progeny at 150 and 200 Gy mean effects of gamma radiation were 16.2 and 8.0 in contrast to control treatment 48.6.

Graham et al., (1972); Flint et al., (1977) reported similar results when both parents treated with radioation doses ranging from 50 to 170 Gy for the pink bollworm. In the present study ANOVA and LSD revealed significant effect at 0.6% on mean number of PTM pupae. The radiation induced sterility of moths due to crosses of treated males with untreated female parents and mating crosses from F1 females and untreated males resulted in percentage reduction of adult counts and oviposition. The Asian corn borer, *Ostrinia furncalis* pupae were irradiated with gamma radiation of 100, 150, 200 and 250 Gy, one or two days before adult emergence revealed F1 sterility and chromosomal aberrations (Zhang et al., 1993).

Gamma radiation effects on PTM adults

Data indicate that the fertility from mating of treated PTM males (100, 150, 200 Gy) doses with untreated females was considerably lower in F2 than F1 mating. This could be explained by the cause of mean effects of eggs production to induced doses as 75.0, 56.7, and 38.3 respectively when compared to control 62.3.

The emerged adults from indirectly affected radiated pupae as a result of different studied PTM crosses demonstrated the sterility induced effects of gamma radiation on mean counts of male and female populations. The 150 and 200 Gy doses showed mean effects on adults were 10.1 and 5.4 respectively as to control treatment 23.4 with a significant difference at 0.01 for F1. On the other hand, F2 adults mean radiation dose effects were 10.6 and 4.7 when compared to control 23.4. For both F1 and F2 progenies the mean irradiation effects on adult PTM moth were 10.3 and 5.1 respectively in comparison to control 23.4. Similar observations were outlined by PTM females and males. Most of these results are in accordance with past studies.

ANOVA and LSD statistical analyses conform to the obtainable data of this study with significance difference at 0.01. The low fertility of F1 progeny of PTM can be attributed to one or more of the following 5 reasons: 1. poor ability to mate; 2. failure to produce as many spermatophores as normal males; 3. Transferred spermatophores that contain little or no sperms; 4. abnormal sperm structure which fails to fertilize the eggs; 5. inheritance of special chromosome rearrangements, (Ashraf and Roppel, 1973; LaChance 1974 and 1985; LaChance et al., 1975; and Anisimov et al., 1989).

In conclusion, results obtained in this study showed that PTM eggs and pupal developmental stages from exposed male parents to gamma radiation doses 100, 150, and 200 Gy and different paired crosses were partially sterilized.

Substerilizing radiation doses in parent generation reduced egg hatching, pupal mortality and lowering male and female adult counts in F1 and F2 generations. These

results are in close conformity with most of those reported in literature for induced gamma radiation techniques for lepidopteran pests as a method of insect pest control.

Experiments on sterility inheritance of PTM radiated at substerilizing doses revealed that F1 generation sterility was higher than male parent generation sterility. In the F2 generation, inherited sterility also noted, at lower rate than in the F1 sterility.

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