IRRADIATION AS A QUARANTINE TREATMENT OF CUT FLOWERS, GINGER AND TURMERIC AGAINST MITES, THRIPS AND NEMATODES

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Abstract

Effect of radiation on different developmental stages of mites, thrips, and nematodes were observed to determine their sterility doses and to develop a method for detection of irradiated and unirradiated specimens. A brief survey on cut - flower and tuber associated pests, and their biological study along with the tolerance level of host products were conducted. Mites *Oligonychus biharensis* (Hirst) and *Tetranychus* sp., as well as four species of thrips viz. *Retithrips syriacus* (Mayet), *Haplothrips gowdeyi* Franklin, *Frankliniella intonsa* Tribom, and *Microcephalothrips abdominalis* Crowford were recognized as common pests damaging plants and cut-flowers. Common species of nematodes infesting ginger and turmeric were *Meloidogyne* spp. and *Ditylenchus* spp. Results indicated that a dose 0.2 kGy and above caused complete sterility of male and female mites and insects. Various preadult developmental stages required less irradiation dose (0.05 - .1 kGy) for sterilization. Variation of melanization in treated and untreated life stages of mites and thrips could not be observed even at 0.2 kGy with the 2 - methyl DOPA spot test. Inhibition of melanization in irradiated pupal stages of thrips were observed at doses above 0.4 kGy. Both irradiated and unirradiated thrips were identical in their protein banding pattern. Virtually no protein bands were observed in irradiated and unirradiated nematodes when samples were run on 5% PAGE in TBE. Tube rose and marigold treated with higher dose (0.3 to 0.5 kGy) caused no remarkable morphological degradation for 7-8 days after irradiation. Nematodes were resistant to radiation. Complete elimination and abnormalities of J_2 stages of *Meloidogyne* spp. and *Ditylenchus* spp. were not observed even at 4.0 kGy although significant weight loss and spoilage of tubers were recorded after 14 days of radiation exposure.

1. INTRODUCTION

Different varieties of cut-flowers grown in Bangladesh have promising economic potential and prospects in international trade. The plants and flowere may be infested with various pests, including mites, thrips, aphids and moths inflicting considerable damage to cash earning crops. Control of these pests are essential for boosting production as well as satisfying quarantine requirements. At present strict and effective quarantine measures are applied to prevent the establishment of pests into areas where they do not occur. Moreover, use of pesticides and fumigants are likely to be restricted in near future because of their residual and hazardous problems. Among the various methods of quarantine requirements, radiation treatment emerged as one of the most suitable and practical alternatives for fresh commodities including cut-flowers. Two species of mites, *Oligonychus biharensis* (Hirst) and *Tetranychus* sp., are most abundant and found throughout the year. Four species of thrips collected from cut - flowers and flowering plants were identified in co-operation with "The Identification Services" of the International Institute of Entomology, London, UK. These species are *Retithrips syriacus* (Mayet), *Microcephalothrips abdominalis* Crowford, *Frankliniella intonsa* Trybom, and *Haplothrips gowdeyi* Franklin. Ginger and turmeric mainly used as spices are infested by various nematodes species causing damage in the field and in post harvest storage. Among various species of nematodes, *Meloidogyne* spp. and *Ditylenchus* spp. are common and widely spread

throughout the country [1] [2]. Before advocating irradiation as a suitable alternative method for cut-flowers, ginger, and turmeric, emphasis should be given on standardization of effective radiation dose and to identify the irradiated insects and mites present in the samples. Several studies have been made to distinguish irradiated insects from unirradiated ones. Rahman *et al.* [3] [4] and Nation *et al.* [5] studied the effects of radiation on the size of supraoesophageal ganglion in relation to the proventriculas in several tephritid fruit flies. Failure of melanization and a spot test for phenoloxidase activity with 2-methyl DOPA was developed by Nation *et al.* [6] for detection of irradiated tephritid fruit flies. Reduced melanization of the confused flour beetle, *Tribolium confusum* following radiation treatment has been observed by Ignatowicz [7]. Radiation sensitive protein markers have been detected in gamma irradiated larvae and pupae of oriental fruit flies by using SDS polyacrylamide gel electrophoresis [8].

Keeping the above views in mind the following investigations have been carried out during the past five years under this project:
(a) Biology and radiosensitivity of mites and thrips infesting cut-flowers.
(b) Determination of tolerance level and vase-life extension of cut-flowers applying radiation doses and low-temperature treatment.
(c) Isolation and culture of nematodes infesting ginger and radiation disinfestation of nematodes along with tolerance level of the plant materials.
(d) Methods for identification of treated insects, mites, and nematodes to determine ways to detect when any stage of these pests found in a particular treated produce have been irradiated.

2. MATERIALS AND METHODS

2.1. Rearing of mites and thrips

The biology and behavior of mites and thrips were studied both in the laboratory and under natural conditions. Two species of mites, *O. biharensis* and *Tetranychus* sp., as well as four species of thrips, *R . syriacus*, *H. gowdeyi*, *F. intonsa*, *M. abdominalis*, were frequently obtained from the experimental garden throughout the year . In case of mites, the biology and radiosensitivity of *O. biharensis* were considered for this study. Among the thrips, *R . syriacus* was the most common species causing maximum damage to rose gardens. These were used as experimental mites and insects and an attempt was made to determine the doses at their various life cycle stages to cause inability to reproduce. Rose leaves were chosen for laboratory rearing of the experimental mites and thrips [9] [10]. The eggs of both species were collected either from infested host leaves or from laboratory reared females. The egg laden host leaves were placed over water soaked cotton in petri dishes. All the life stages of mites were reared in the laboratory on rose leaves placed over water soaked cotton. The eggs of thrips were reared similarly but larvae, pupae, and adult thrips were cultured on rose leaves whose main stalks were usually inserted in wet cotton stubs placed in small plastic vials. The whole sets were kept in a glass beaker covered with fine meshed nylon net. Laboratory rearing were maintained at ambient temperature (25 $\pm$ 5°C, 60 $\pm$ 10% R.H.). The mites and thrips were transferred to fresh leaves from time to time to ensure food supply. The different life stages of the mites and thrips were observed under a binocular dissecting microscope.

2.1.1. Irradiation of mites and thrips

Radiosensitivity and reproductive efficiency of mites and thrips were determined after irradiating different developmental stages of *O. biharensis* and *R . syriacus* with doses ranging from 0.01 to 0.5 kGy for eggs, larvae, pupae, and adults. The crosses between irradiated female deutonymphs and normal males, and irradiated males with normal female deutonymphs were conducted to determine doses to cause inability of reproduction in *O. biharensis*. Sterility doses for males and females *R.. syriacus* were determined from the success of the F_1 generation after crossing

between treated and untreated adults. Sterilizing doses of developmental stages were determined from the unsuccess of the treated life stages up to adult transformation. Irradiation was from a Co^{60} source at a dose rate of 0.33 kGy / hr.

2.2. Detection of irradiated mites / insects.

2.2.1. Melanization spot test

Phenoloxidase activities in larvae, nymphs / pupae, and adults of *O. biharensis* and *R. syriacus* were determined with the help of the 2 - methyl DOPA test. Twenty mg of 2-methyl-DOPA was dissolved in 10 ml of sodium phosphate buffer (pH 7.2) and 50 µl of this solution containing 100 µg of the substrate was air dried on a non-absorbent transparent sheet. Samples of 50 irradiated and unirradiated larvae, nymphs / pupae and adults were crushed separately in one drop of tap water and the crushed sample was placed by micropipette on each of the dried spots of 2-methyl DOPA in order to observe any change of color. Radiation doses ranging from 0.01 to 0.2 kGy were applied to the biological organisms for this purpose. Morphological deformation and color change, if any, were observed after radiation exposure (0.01 - 0. 5 kGy) to detect the radiation treated species.

2.2.2. Electrophoresis

Electrophoresis was conducted on 5% PAGE in TBE buffers. Test samples were different stages of the life cycle of *R. syriacus*. The samples were irradiated at 20, 40, and 50 Gy. Four to five specimens from each stage were squashed in TBE and bromphenol blue 24 hr. after irradiation. These were run on a vertical slab gel apparatus at 240 V and stained with 0.5% Coomassie Blue. Samples of fruit fly pupae irradiated (50 Gy) at larval stage were run with them for comparison.

2.3. Disinfestation and vase life extension of cut flowers by irradiation and low temperature

Freshly cut marigold and tube rose were collected from the experimental garden and twelve bunches containing 10 flowers of each type were taken for studies. Each bunch of flowers was put inside a perforated polythene pouch and with a cotton plug. From time to time water was sprinkled on the cotton plug with a syringe. Two sets containing six bunches of each type of flower were prepared and irradiated at doses of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy. One set of irradiated flowers was kept at fixed temperature (15°C and 40 $\pm$ 5% R.H.) in an incubator, and the second set at ambient temperature (25 $\pm$ 5°C, 60 $\pm$ 10% R.H.) inside a glass box with a netted door. Observations were made regularly at 24 hr. intervals regarding pest incidence and storage ability of flowers.

2.4. Rearing of nematodes

Nematodes were maintained on fresh and uninfested ginger collected from the experimental garden. The uninfested ginger was innoculated with nematodes in the laboratory either by placing small amount of infested ginger or by few drops of water containing nematodes on the fresh ginger. After three days of intermixing, the gingers were stored in moist condition in petri dishes at the ambient temperature (25 $\pm$ 5° C and 60 $\pm$ 10% R.H.) for a period of 3 months.

2.5. Effect of radiation on ginger

Infested and uninfested ginger packed in 200g polythene bags were used to determine radiation sensitivity. Observations on the extent of damage, occurrence of nematodes, weight loss, and physical appearance of the experimental items were made at different intervals after treatment. A small piece of ginger was placed into 10 ml of water and shaken well for nematode collection. The collected nematodes were kept in distilled water for radiation treatment.

2.6. Irradiation of nematodes

Nematode infested and uninfested ginger was irradiated at doses ranging from 0.0 to 2.0 kGy. The effects of radiation on the J_2 stage of *Meloidogyne* spp. was determined from exposure of infested ginger with doses ranging from 0.0 to 4.0 kGy at a dose rate of 1.2 kGy / hr. Nematodes were collected and kept in distilled water prior to irradiation at the same dose to observe abnormalities and damage.

2.7. Detection of irradiated nematodes

Electrophoresis procedures for nematodes (*Meloidogyne* spp.) were similar to those used with thrips and fruit flies. Five specimens (J_2 stage) from irradiated and unirradiated samples were collected from the laboratory culture and squashed for this study. The sample was placed on the gel and electrophoresed.

3. RESULTS AND DISCUSSION

The life-cycle of the mites, *O. biharensis* and *R . syriacus,* was completed within 20 - 22 days from egg to adult at 25 $\pm$ 5° C and 60 $\pm$ 10% R.H. *O. biharensis* has five distinct stages in their life-cycle, the egg, larva, protonymph, deutonymph, and adult. *R . syriacus* completed its life-cycle in four stages, the egg, larva, pupa, and adult. There was only one larval instar in *O. biharensis, but* two larval instars were present in *R . syriacus*. The pupal stage in *R . syriacus* could be divided into two sub stages, pupa - I of one day duration and pupa - II with two days duration prior to adult emergence. *O. biharensis* laid eggs on both surfaces of leaves, and the thrips *R. syriacus* laid eggs inside the host tissue below the epidermal layer. The incubation period of both mite and thrips was about 8 days and the larval period / instars continued for 3 - 7 days. The nymphal and pupal period was completed in 4 - 6 days. Adult longevity varied from 20 - 30 days in the laboratory. *O. biharensis* and *R . syriacus* were capable of reproducing both sexually and parthenogenetically. The progenies from fertilized eggs were all females and males were produced parthenogenetically. Based on the mode of reproduction, it is believed that if the sex chromosome of the male is inactivated by a suitable dose of radiation, males that mate with normal females will give rise to unfertilized eggs resulting in only males in the F_1 generation. On the other hand, a female deutonymph treated with a sub lethal dose and crossed with normal males will either produce both males and females or will not lay any viable eggs if exposed to a sterilizing dose. Deutonymphs were utilized in this experiment only to ensure their virginity prior to desired pairings.

3.1. Male mite sterility

The irradiated males were apparently sexually active even after treatment with 0.5 kGy. They guarded the female deutonymph and mated with them immediately after adult emergence. An average of four eggs were laid by control females per day, but egg laying totally ceased during the last 2-3 days of adult life. From this cross an average of 21 females and two males were obtained in F_1 generation, giving a sex ratio of about 9:1 in the adult population. The average number of eggs laid by the normal female paired with treated males ranged from 12 - 31 (Table I). Longevity of males was about 13, 15, 13, 9, and 8 days, and hatchability of eggs was 72%, 48%, 33%, 29%, and 15% in the crosses of normal females with males treated with 0.1, 0.2 , 0.3, 0.4, and 0.5 kGy, respectively. The sex ratio started to become male biased from the normal 9:1 female to male ratio to 1:3 when 0.1 kGy treated males were crossed with normal females. Total cessation of production of F_1 females resulting from pairing of normal females with males irradiated with 0.2, 0.3, 0.4, and 0.5 kGy, producing only 8.6%, 16.6%, 14.3%, and 15.1% male progeny, respectively. It is apparent from the results that treating males with 0.2 kGy and above made them sterile, resulting in subsequent production of male progeny in the F_1 generation.

TABLE I. EFFECT OF RADIATION ON CHANGING SEX RATIO AND INDUCED STERILITY IN THE F_1 GENERATION IN *O. BIHARENSIS* WHEN CROSSES WERE MADE BETWEEN TREATED MALES AND UNTREATED FEMALES, AND UNTREATED MALES AND TREATED FEMALES.

Dose (kGy)	Untreated female x treated male				Treated female x untreated male			
	Mean ± (S.D.) eggs laid	Mean ± (S.D.) adult emergence		Male sterility	Mean ± (S.D.) eggs laid	Mean ± (S.D.) adults produced		Female sterility
		Females (F_1)	Males (F_1)			Females (F_1)	Males (F_1)	
0.0	25 (7.9)	21.0 (1.6)	2.0 1.16	None	16 (3.7)	9 (1.6)	1 (0.7)	None
0.1	25 (7.6)	1.5 (0.9)	4.5 (0.8)	Almost all	18 (4.5)	5 (1.6)	1 (1.22)	Some
0.2	31 (4.4)	Nil	2.5 (1.2)	All	6 (1.6)	1 (1.22)	Nil	Almost all
0.3	12 (3.2)	Nil	2 (1.0)	All	4.5 (1.9)	Nil	Nil	All
0.4	14 (2.92)	Nil	2 (1.0)	All	3.0 (1.6)	Nil	Nil	All
0.5	16.5 (5.7)	Nil	2.5 (1.5)	All	5 (2.7)	Nil	Nil	All

Female mite sterility : No effect on adult emergence from the deutonymphal stage to adult has been observed up to 0.3 kGy. About 50% eggs hatched in 0.1 kGy treated females compared to 100% egg hatch in control pairs. The average number of eggs laid by females treated with 0.2, 0.3, 0.4, and 0.5 kGy when crossed with normal males were 6.0, 4.5, 3.0, and 5.0 eggs, respectively. All eggs laid by females treated with 0.3 kGy failed to hatch. It appeared that the sterility dose for adult females lies within 0.2 kGy. This result also agrees with the sterility dose of 0.25 kGy for male and female mites as reported by Ignatowicz [11].

Mite egg sterility : All control eggs hatched and passed through the subsequent developmental stages to become adults, except 5.9% due to factors other than radiation. Egg hatchability gradually decreased from 90.5 - 76.4%, resulting in still lower percent of adults (79.7 - 38.0%) when treated with 10.0 - 50.0 Gy. Only a few percent (6.0 - 3.4%) of eggs hatched after exposure to $\geq$ 60.0 Gy, and all of them failed to reach the adult stage.

3.2. Radiosensitivity of thrips

Table II indicates the effects of radiation on eggs, larvae, pupae, and adults of *R . syriacus*. Eggs (5 - 6 days) treated with 0.1 kGy showed some sort of development initially, but failed to be transformed into the adult stage. The early eggs (0 - 24 hr.) of *R . syriacus* irradiated with 0.1 kGy were damaged, and without further development. Larvae (I and II) treated with 0.1 kGy and above also did not to develop to the following life stage. A few adults were obtained from late eggs and larvae exposed to radiation dose of 0.05 kGy, whereas most of the unirradiated eggs and larvae successfully developed into adults in the laboratory. Pupae (I and II) exposed to a dose of 0.01 - 0.25 kGy showed 86 to 99% adult emergence. Less than 50% adult emergence of the above two stages were observed when treated with $\geq$ 0.3 kGy, and adult emergence totally stopped after exposure of $\geq$ 0.5 kGy. Eggs laid by emerging adults that were treated with $\geq$ 0.15 kGy failed to develop into adults. When adult males and females (1 - 2 days age) were exposed to an irradiation

TABLE II. ADULT *R. SYRIACUS* EMERGENCE AND STERILITY OF ADULTS AFTER VARIOUS DEVELOPMENTAL STAGES WERE TREATED WITH DIFFERENT DOSES OF GAMMA RADIATION.

Dose (kGy)	Egg (5-6 days)		Larva I + II		Pupa I + II		Adult (1-2 days)	
	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% Sterility ± S.E (Male)	% Sterility ± S.E (Female)
0.0	86.0 (1.4)	0.0	94.0 (2.5)	0.0	100	0.0	0.0	0.0
0.01	81.0 (1.87)	6.3 (1.08)	85.3 (1.78)	4.3 (1.08)	99.3 (2.12)	2.6 (1.08)	7.0 (1.41)	2.6 (0.81)
0.025	65.0 (1.9)	44.3 (2.16)	68.6 (2.95)	26.3 (2.55)	96.6 (1.47)	13.6 (2.49)	27.0 (3.59)	22.0 (2.27)
0.05	24.0 (1.87)	88.6 (2.40)	40.0 (2.16)	693 (2.95)	98.6 (1.08)	66.6 (4.3)	68.6 (3.56)	64.0 (2.1)
0.10	0.0	100	0.0	100	97.3 (1.5)	83.6 (3.9)	90.0 (4.96)	85.0 (3.25)
0.15					98 (1.41)	100	100	100
0.20					94.0 (2.55)			
0.25					86.0 (1.41)			
0.30					49.0 (2.12)			
0.35					31.3 (2.95)			
0.40					17.3 (2.95)			
0.45					4.3 (1.78)			
0.50					0.0			

dose of 0.15 kGy and paired, the females laid 15-20 eggs but they did not hatch. In controls the total number of eggs laid by a pair within the life span varied from 18 - 25. A dose for eggs and larvae that results in inability to reproduce was 0.10 kGy, whereas a higher dose of 0.15 kGy was required for pupae and adults.

3.3. Identification of irradiated insects / mites

Pupae I and II of *R. syriacus* are reddish in color and the adults are black in general appearance. The enzyme responsible for blackening the body of the adults apparently starts in the early pupa II stage. None of the pupa I attained adulthood, although some of them were transformed to pupa II stage even after exposure of 0.5 kGy. Pupae II that developed from pupae I treated with 0.5 kGy were always reddish in color. However, pupae II that transformed from pupae I treated with 0.4 kGy showed blackening in about 50% of the individuals, while the rest appeared reddish without showing any sign of melanization. Thus, in *R. Syriacus* thrips, the occurrence of reddish pupae I and II in radiation treated floricultural produce is an indicator of their exposure at least to a dose of 0.5 kGy.

The 2-Methyl DOPA test for larvae (I and II), pupae (I and II), and adults of *R . syriacus* was negative, and did not produce the red color turning to black color that indicates phenoloxidase. Third instars of the tephritid fruit fly, *Bactrocera cucurbitae,* did give a positive test, turning the 2-methyl DOPA spot black. Second instars of *B. Cucurbitae* irradiated with 0.04 kGy transformed into third instars, the spot test with such a third instar was negative indicating the lack of phenoloxidase after the radiation treatment. These results agree with those found in other tephritid fruit flies [6] [12]. The 2-methyl DOPA test for larvae, protonymphs, deutonymphs, and adults of *O. biharensis* was negative for both control and irradiated samples. All the stages of irradiated and unirradiated *R.*

syriacus were identical in their protein banding pattern, whereas in the fruit flies the banding patterns in the irradiated samples were different from those of the controls as indicated by the absence of a protein band in pupae.

3.4. Vase life of flower

No difference could be detected between the control and irradiated samples of marigold and tube rose after irradiation with respect of physical condition, color, and odor. All flowers treated up to a dose of 0.5 kGy were acceptable for keeping in the vase for 7 - 8 days under laboratory conditions, but flower bunches kept at low temperature (15° C, 40 ± 5% R.H.) were in good condition for about 12 - 14 days. About 8 days after radiation treatment (0.5 kGy), flowers became shrunken with curling of petals, and black spots started to appear on the sepals. A large number of mites and thrips, along with other pests, were observed in the polythene bag containing control flowers at ambient temperature. Thus, irradiation and low temperature treatment seemed to be effective for disinfestation and shelf-life extension of marigold and tube rose.

3.5. Nematodes infesting ginger

The contaminated ginger showed heavy infestation of nematodes after 2 - 3 weeks. Both irradiated and unirradiated ginger contained various species of nematodes. Among the ematodes, *Meloidogyne* spp. and *Ditylenchus* spp. were most common, but there also were other species. Rotting, blackening at the budding zones, and lesions were observed. Table III represents the mortality of second stage of Juveniles (J_2) of *Meloidogyne* spp. in ginger at different intervals after exposure to 0.0 kGy to 4.0 kGy gamma radiation. Results after 14 days indicated that only 34.2% reduction of J_2 stages was obtained at 4.0 kGy. Significant deterioration in color and texture was observed at 1.0 kGy and above. Most of the sample irradiated above though the dose level affects the quality of ginger within this period. Virtually no protein band and deformities were observed in irradiated and unirradiated nematodes when samples were run on 5% PAGE in TBE and were observed under the microscope. Table IV represents loss of weight of control and irradiated gingers in ambient temperature at different intervals. In controls the average weight loss was 67% after 90 days. In contrast the weight loss in irradiated samples was quicker and higher. Significant deterioration in color and texture was observed at 1.0 kGy and above. Most of the samples irradiated above 1.0 kGy became brittle after 45 days of observations. After 30 days, these became unacceptable for marketing and human consumption. Because of deterioration of the qualities of the product, the highest dose (4.0 kGy) was not applied for storage studies.

TABLE III. MORTALITY OF J_2 STAGES OF *MELOIDOGYNE* SPECIES OF NEMATODES IN GINGER AT DIFFERENT DOSES OF RADIATION.

Dose (kGy)	Mean percentage of mortalities on different days ± (S.E.)							
	0	2	4	6	8	10	12	14
0.0	-	0	1.26 (0.10)	2.5 (0.18)	3.3 (0.21)	3.6 (0.14)	5.13 (0.21)	6.5 (0.69)
1.0	-	0	1.86 (0.21)	2.6 (0.12)	4.96 (0.42)	6.8 (0.42)	6.13 (0.49)	9.13 (0.55)
2.0	-	1.12 (.11)	1.93 (0.17)	2.6 (0.12)	6.26 (0.29)	8.53 (0.43)	12.2 (0.35)	16.33 (0.91)
3.0	-	1.60 (0.14)	2.23 (0.22)	5.73 (0.49)	7.5 (0.21)	14.93 (0.29)	17.73 (0.80)	29.4 (0.65)
4.0	-	2.0 (0.24)	2.63 (0.17)	6.06 (0.29)	11.86 0.67	16.53 0.85	18.26 (1.56)	34.2 (1.43)

TABLE IV. PERCENTAGE OF WEIGHT LOSS OF IRRADIATED AND UNIRRADIATED GINGER AFTER THREE MONTHS OF STORAGE AT AMBIENT TEMPERATURE.

Doses (kGy)	Mean percentage of weight loss after varying storage time in days ± (S. E.)						
	0	15	30	45	60	75	90
0.0	-	21.6	29.9	41.5	56.8	62.4	67.6
		(5.8)	(3.6)	(6.3)	(7.4)	(3.8)	(4.4)
0.50	-	29.4	45.8	62.4	60.2	74.4	77.3
		(4.7)	(6.4)	(5.8)	(2.8)	(2.2)	(2.2)
1.00	-	35.6	52.5	70.4	74.6	89.4	90.4
		(4.9)	(6.6)	(5.4)	(4.2)	(2.0)	(1.2)
1.50	-	37.4	60.4	71.3	80.2	89.4	91.3
		(5.5)	(6.7)	(6.6)	(4.4)	(1.2)	(1.2)
2.00	-	31.6	63.3	87.5	91.2	91.8	92.0
		(4.7)	(4.1)	(4.6)	(2.4)	(1.2)	(0.88)

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