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RADIATION-HEAT SYNERGY COMBINED WITH FUNGICIDES IN POST-HARVEST PATHOLOGY

By

M. K. Roy
Nuclear Research Laboratory
Indian Agricultural Research Institute
New Delhi- 11012

In vitro studies with fungi namely *Phytophthora parasitica*, *Aspergillus niger* and *Fusarium coeruleum* revealed that the dose of 150 krad of gamma radiation has very little fungicidal effect on the growth of *A. niger* and *F. coeruleum* while the growth of *P. parasitica* is completely prevented by this dose of radiation.

In vivo studies with apples showed that the decay of the fruits due to 48 hour old infections of *Penicillium expansum* could be completely prevented by double combination of 250 ppm benomyl preceded by hot-water (50°C/5mts) at an interval of 1/2 hour or by the following sequences of triple combination of 50°C/10 mts —150 krad of radiation—250 ppm benomyl at 56°C/4 mts—150 krad of radiation—1000 ppm aureofungin. The triple combination of hot-water (50°C/10 —mts) 150 krad—1000 ppm benomyl completely controlled the 2 day old infections of the fruit by *Botrytis cinerea*. This decay of apples due to *A. niger* was completely prevented by hot-water at 50°C/10 mts followed by 150 krad of radiation or by the triple combination of hot-water-irradiation and fungicides (1000 ppm aureofungin or 250 ppm benomyl) during the 3-weeks holding period at 25°C. Results of combined treatments in the control of *P. parasitica* inoculated in tomatoes have also been presented in the paper.

Pre-inoculation dips of apples in aureofungin, benomyl were either ineffective or partially effective in preventing subsequent rots of the fruits by *B. cinerea*, *P. expansum*, *Alternaria tenuis* and *A. niger*. Evidence obtained also showed that the double combination of hot-water and irradiation did not possess any preventive property against these fungal infections.

The organoleptic scores according to the 9 point Hedonic scale, of apples treated with hot-water and irradiation were dependent on variety, temperature and period of storage, when the treated fruits were evaluated 3-4 hours after treatment or 3 weeks after holding at 10°C their taste test scores compared favourably with controls. Some loss in texture or taste was observed, however, after apples were held at 25°±1°C for 3 weeks.

INTRODUCTION

Radiation when combined with hot-water inactivates synergistically the spores of a number of fungi and offers new a potential for the cure of fungal infections latent and patent in fruits and vegetables (Barkai-Golan *et al.* 1969. Benarie and Barkai-Golan 1969, Sommer *et al.* 1967, 1968; Brodrick *et al.* 1976). Like low temperature storage and chemical surface disinfectants doses of gamma radiation within the tolerance limits of hosts can provide only partial control of rots. The dose of 150 krad of radiation is the tolerance limit of apple and tomato fruits (Maxie, *et al.* 1971). Hot-water alone has been attempted with but limited success to control post-harvest pathogens of several fruits including apple and tomato (Sharples 1967; Rosenbaum 1920). A combination of heat-irradiation has the added advantage that the problem of chemical residues does not arise. The use of chemical fungicides may be desirable in protecting fruits and vegetables from subsequent infection. In tomatoes of comparatively short physiological life (3-7 days) this combination of heat-irradiation may show good promise extending the shelf-life (10 days or more) as radiation delays ripening and senescence also. No information is available on the organoleptic changes in fruits due to the combined treatment of hot-water and radiation.

MATERIALS AND METHODS

The methods for fungal inoculation of the fruits, fungicidal dips, hot-water treatment, spore-counts have been described (Roy and Mukewar 1972, Roy 1975). The growth in diameter of the rot was measured every week to record the degrees of infection and extent of control. Apparently sound and uninjured apples of Royal Delicious and Ambri varieties were used for the studies. The organoleptic evaluation was based on the 9-point hedonic scale..... 9 is 'like extremely'; 1 is dislike extremely; 5 is 'neither like nor dislike' and scores of 5.0 and above are considered acceptable (Anonymous 1970). Storage of fruits

after treament was at 25 + 1°C. In all experiments irradiation followed hot-water at intervals of 3/4 —2 hours, whereas the interval between heat and fungicide or irradiation-fungicide was about 30-40 minutes. Irradiation followed hot-water but preceded fungicide; hot-water also preceded fungicide in double combination. The sequence of component treatments in triple combination was hot-water-irradiation and fungicidal dips. 5-10 fruits were used for each treatment and experiments containing all the treatments were repeated at least thrice. Suitable controls were always maintained.

EXPERIMENTAL

In Vitro studies :

one-day old mycelia of *A. niger*, *F. coeruleum* and *P. parasitica* on PDA irradiated with various doses of radiation were held at 25±1°C or 32±1°C. The data on growth diameter recorded in Table 1 show that *P. parasitica* is much more radiosusceptible than *A. niger* and *F. coeruleum*. Its growth is completely stopped by irradiation at 150 krad, That radiation delays the growth of *A. niger* and *F. coeruleum* was observable at doses 100 krad onwards but this effect was fast nullified subsequently as indicated by the increased diameter of the colony with passage of time.

Pre-inoculation experiments

Preliminary studies showed that dips of apples in 1000 ppm aureofungin were partially effective against *A. niger* and in-effective against *P. expansum*, *B. cinerea* and *A. tenuis* ; while dips of 1000 ppm benomyl were partially effective in preventing subsequent infection of the fruits by these fungi. The treatment, prior to inoculation of apples with the double combination of hot-water (50°C/10 mts. or 56°C/4 mts.) and 150 krad of radiation could not provide for protection(medium or indirect effect) of the fruits from later infection by the fungus at about 25°C.

Table 1. *Comparative growth (averages of 3 replications) of 24 hour old mycelia of* F. Coeruleum, P. parasitica *and* A. niger *after irradiation*

Doses	Growth (diameter of colony in cm. indicated number of days) *F. coeruleum* ++				*P. parasitica*++				*A. niger*			
	1	3	5	8	1	3	6	11	1	2	3	4
						Traces						
1. 50 krad					0.0	—3.5	8.0					
2. 100 krad	1.2	3.6	5.7	7.0	0.0	0.0—1.5	0.0—2.5	0·0—5.5	2.0	2.8	3.7	4.3
3. 150 krad					0.0	0.0	0.0	0.0	1.4	2.6	4.1	4.3
4. 200 krad	0.8	2.7	5.2	7.0	0.0	0.0	0.0	0.0	1.0	2.7	4.0	4.4
5. 250 krad									1.0	2.1	3.9	4.6
6. 300 krad	0.8	1.5	3.9	7.0								
7. 400 krad	0.9	1.6	3.8	7.0								
8. Control	0.9	2.8	4.9	7.0		2.3	4.6	8.5	1.5	3.0	4.0	4.6

+ Growth in 5.0 cm Petriplates at 32±1°C

++ Growth in 8—10 cm Petriplates at 25±1°C.

In vivo studies :

Data presented in Tables 2, 3 and 4 show that all the treatments were ineffective singly in the control of 48-hour old infections of apples at about 25°C. Hot-water at 50°C for 10 minutes coupled with 150 krad of radiation gave completed control of *A. niger* and 80-100 per cent control of *B. cinerea* and

Table 2. *Benomyl, 1000 ppm aureofungin singly and in combination with hot-water (50°C/10 minutes), irradiation (150 or 100 krad) in the control at 25+1°C of 48 hour old infections of A.* niger *in apples*

	Progress of infections and extent of rot control in per cent after indicated number of days.					
	7		14		21	
	Degrees* of rot	Extent of control	Degrees of rot	Extent of control	Degrees of rot	Extent of control
1. Hot-water	1—2	0	3	0	4	0
2. Irradiation (150 krad)	0—2	20	3	0	4	0
3. Aureofungin	0—2	40	3	30	4	0
4. Benomyl 250 ppm	0—1	20	2	0	3	0
5. Hot-water + Aureofungin	1—2	0	3	0	4	0
6. Hot-water + 100 krad irradiation	1—2	70	2—3	50	3—4	30
7. Irradiation (150 krad) +Aureofungin	0—2	20	3	0	4	0
8. Hot-water+Irradiation (150 krad)	0	100	0	100	0	100
9. Hot-water + 1000 ppm + benomyl	0—1 1	60—80 60	0—2 2	60 50—60	0—3 3	60 50
10. Hot-water + 250 ppm benomyl	0—1	60	0—2	50	0—3	50
11. Hot-water + irradiation (150 krad) + Aureofungin	0	100	0	100	0	100
12. Hot-water + 150 krad irradiation + 250 ppm benomyl	0	100	0	100	0	100
13. Control	2	0	3	0	4	0

* Degrees of rot : 0 = No progress of infection beyond inoculated area;
1 = Diameter of rotten area upto about 1.5 cm;
2 = Diameter of rotten area upto about 2.5 cm;
3 = Diameter of rotten area upto about 3.5 cm;
4 = Diameter of rotten area upto about 3.5 cm

+The time interval between treatments was 5—7 hours.

Table 3. *Combined double and triple combination treatments of radiation (150 krad), heat and 1000 ppm aureofungin and benomyl in the control of 48 hours old infections of* P. expansum *in apples at 25 ± 1°C.*

Treatments	Quantity of inoculum	Progress of infection and extent of rot control in per cent after indicated number of days					
		7		14		21	
		Degrees of rot	Extent of control	Degrees of rot	Extent of control	Degrees of rot	Extent of control
1. 50° C/10 min. Undefined & 150 krad radiation & 250 ppm benomy 1		0	100	0	100	0	100
2. 50° C/5 min. Undefined & 250 ppm benomy 1		0	100	0	100	0	100
3. 50° C/10 min. Undefined		0—1	80	0—2	80	0—3	80
& irradiation Defined *		0	100	0	100	0—1	80
4. 56° C/4 min. Undefined		0—1	80	0—2	80	0—3	80
& Irradiation Defined		0	100	0	100	0	100
5. 50° C/10 min. Undefined & aureofungin		2	0	3	0	4	0
6. Irradiation Undefined		2	0	3	0	4	0
& Aureofungin Defined *		2	0	3	0	4	0
7. 50° C/10 min. Undefined		0—2	80	0—3	80	0—4	80
+ Irradiation Defined +		0	100	0	100	0	100
+ Aureofungin							
8. 56° C/4 min.							
+ Irradiation Undefined &		0	100	0	100	0	100
+ Aureofungin Defined + +							
Control	Defined & undefined	2	0	3	0	4	0

* Defined — 2×10^4 spores/infection.

+ + Defined — $2 \times 10^{4-5}$ spores/infection.

P. expansum. The triple combination of hot-water-irradiation-1000 ppm aureofungin, 250 ppm benomyl also completely controlled the 2-day old infections of the fruits by *A. niger*. This decay of the fruits by *P. expansum*

could be completely controlled by a double combination of 250 ppm bnomyl preceded by hot-water (50°C/5 mts) or by the following sequences of triple combinations namely 50°C/10 mts. -150 krad -250 ppm benomyl or 56°C/4mts. —150 krad - 1000 ppm aureofungin. A complete control of *B. cinerea* was obtained by 50°C/10 mts - 150 krad - 1000 ppm benomyl.

Table 4. *Benomy 1, aureofungin (1000 ppm) alone and in combination with hot-water (50°C/10 mts.), 150 krad fo radiation in the control at 25°C of 48-hour old infecrions of* B. cinerea *in apples*

Treatments	progress of infection and extent of rot control in per cent after indicated number of days					
	7		14		21	
	Degrees of rot	Extent of control	Degrees of rot	Extent of control	Degrees of rot	Extent of control
1. Hot-water	1—2	0	3	0	4	0
2. Irradiation	0—2	20	3	0	4	0
3. Aureofungin	2	0	3	0	4	0
4. Benomyl 250 ppm	0—2	80	0—3	50	0—4	50
5. Benomyl 1000 ppm	0—2	80	0—3	70	0—4	70
6. Hot-water + Aureofungin	0—2	30	0—3	30	0—4	30
7. Hot-water + 250 ppm benomyl	0—2	70	0—3	60	0—4	60
8. Hot-water + 1000 ppm benomyl	0—2	80	0—3	60	0—4	60
9. Irradiation + Aureofungin	1—2	0	3	0	4	0
10. Irradiation + 1000 ppm benomyl	0—1	50	0—2	50	0—3	50
11. Hot-water + irradiaton	0—1	80	0—2	80	0—3	80
12. Hot-water + Irradiation + 1000 ppm benomyl	0	100	0	100	0—1	100

Data on tomatoes in Table 5 show that the double combination of hot-water at 55° for 6 mts, and 50 or 100 krad of radiation gave control at 25°C ±1°C of 24-hour old infections in tomatoes by *P. parasitica*.

Table 5. *Combined double combination of heat-irradiation in the control at 25 ± 1°C of 24 hour old infections of* Phytophthora parasitica *in tomatoes*

Treatment	Progress of infection and extent of rot control after indicated number of days					
	3		7		10	
	Degrees of rot	Extent of control	Degrees of rot	Extent of control	Degrees of rot	Extent of control
1. 55°C/6 mts.	0—2	80	0—3	60	0—4	50
2. 55°C/6 mts. + 50 krad	0	100	0	100	0—1	80
3. 55°C/6 mts. + 100 Krad	0	100	0	100	0	100
4. 100 krad	0—1	85	2—3	60		
5. Control	2—3	0	3	0	4	0

Organoleptic scores :

The apples were treated singly with radiation (150 krad), hot-water at 50°C for 10 mts. or 56°C for 4 mts, or their combinations. The data on taste test scores by 5-7 penalists revealed that when apples treated with hot-water and irradiation are tasted 3-4 hours after treatment or after storage for 3 weeks at 10°C, the average rating scores on color, texture and flavour were acceptable, but the two varieties used differed in their scores and when stored at 25±1°C the scores were either maginally acceptable or were below the level of acceptance (Table 6).

Table 6. *Organoleptic evaluation of Royal Delicious and Ambri apples treated with hot-water and irradiation*

Treat—ments	Time interval between treat—ment and evaluation	Variety of apple	Average sensory scores on colour	Flavour (taste or aroma)	Texture
T*$_1$	3—4 hours	Ambri	7.3	7.2	7.3
T$_3$	—	Ambri	7.3	7.6	7.5
T$_2$	3—4 hours	Ambri	7.5	7.0	7.6
T$_1$	3—4 hours	Royal Delicious	7.2	7.0	8.0
T$_3$	—	Royal Delicious	8.1	6.8	8.0
T$_2$	3—4 hours	Royal Delicious	7.0	7.2	7.3
T$_5$	21 days	Ambri	5.7	5.0	5.4
T$_5$	21 days	Royal Delicious	5.7	5.5	6.0
T$_4$	21 days	Ambri	5.0	5.3	5.2
T$_4$	21 days	Royal Delicious	6.2	5.0	5.7
T$_2$	15 – 22 days	Ambri	5.6	5.5	5.4
T$_3$	— do —	— do —	6.0	6.0	5.5
T$_2$	— do —	Royal Delicious	6.5	4.5+	5.2
T$_1$	— do —	Ambri	5.6	5.0	4.2+
T$_1$	— do —	Royal Delicious	5.8	5.0	5.0
T$_3$	— do —	— do —	6.2	5.8	5.8
T$_2$a	21 days	Ambri	5.7	6.8	6.4
T$_3$b	— do —	— do —	8.0	6.7	7.8
T$_2$c	— do —	Royal Delicious	8.0	6.6	6.0
T$_3$d	— do	— do —	8.0	7.0	7.2

a —d : Storage temperature after treatment was 10°C and in other cases this temperature was 25±1°C.

+ unacceptable scores

* T$_1$ — Hot water at 56°C/4 minutes + 150 krad irradiation.

T$_2$ — Hot water at 50°C/10 minutes + 150 krad irradiation.

T^3 — Control, T$_4$ — Hot water at 50°C/10 minutes

T$_5$ — Hot water at 56°C/5 minutes.

DISCUSSION

It is known that Beraha, Smith & Wright, 1960) *Phytophthora infestarns* and *Phoma citri* are extremely radiation sensitive. Next to them here is the first report of *Phytophthora parasitica* which falls into the class of fungi moderately sensitive to radiation while F. *coeruleum* into the resistant class. The

results presented here also indicated the variability in radiation sensitivity of the fungi within the genus *Phytophthora*. A dose of 100 krad of gamma radiation or the hot-water treatment at 55°C for 6 minutes when attempted singly was only partially effective but in combination these could completely control the infections in tomato. This indicated, on calculation, synergistic interaction between heat and irradiation. Similar studies are also in progress with other fungi.

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