Nematol. medit. (1999), 27: 225-231

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EFFECT OF MICROWAVE IRRADIATION ON THE EAR-COCKLE DISEASE AND YIELD OF WHEAT

by
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Summary. Microwave irradiation significantly decreased the mycoflora of soaked (from 8 and 10 sec. onwards) and unsoaked (from 10 and 12 sec onwards), healthy and cockled seeds, respectively. Mortality of *Anguina tritici* juveniles inside soaked and unsoaked cockles was significantly greater from 10 and 14 sec treatments, respectively. The seed germination decreased (P=0.05) from 16 sec onwards. Microwave treatments gradually suppressed the pathogenic effect of *A. tritici*, leading to a significant increase in the growth and yield of inoculated plants grown from 14 or 16 sec exposed healthy seeds (soaked). Ear-cockle or seed-gall formation was also decreased (P=0.05). Microwave exposures for 18 sec onwards, however, caused injurious effects that resulted to a significant decrease in the plant growth and yield of wheat.

Ear-cockle of wheat caused by the seed-gall nematode, *Anguina tritici* (Steinbuch) Chitw. is an important disease of wheat in Asia, including India (Swarup and Sosa-Moss, 1990). Recently, Khan and Athar (1996) reported 6-18, 12-28 and 24-43% decrease in the yield of different cultivars of wheat at 15000, 20000 and 30000 J_2/plant, respectively.

Galls are the sole source of nematode inoculum. They are sown in the field with healthy seeds. Hence, a device that could separate cockles from the healthy seeds or kill the juveniles inside the cockle can effectively reduce the disease incidence and its severity. Mechanical sieving, flotation of contaminated seeds in brine (diluted NaCl solution) or hot-water treatment have been used successfully to eliminate the nematode from Europe and other areas (Brown and Kerry, 1987). In India, farmers do not feel confident to implement these techniques which are manual and involve personal error. Further they do not completely eliminate cockles.

The present investigation explores feasibility of microwave irradiation to control the ear-cockle disease of wheat (*Triticum aestivum* L.).

Materials and methods

A microwave oven, 980 Watts, generating microwaves at a frequency of 2450 MHz/sec with a rotatory platform (Batliboi Eddy, ER-5054D, India) was used to expose cockled or healthy seeds of wheat cv. HD 2009 which were first soaked with distilled water for 6 hours or unsoaked.

Healthy and cockled wheat seeds, soaked with water or unsoaked were exposed to microwave irradiation for 0, 4, 8, 10, 12, 14... 30 sec in sterilized Petri plates. After exposure, the seeds were placed on potato dextrose agar in

Petri plates (10 seeds/plate, 10 plates/treatment), and were incubated at 25 °C in a Biological Oxygen Demand (B.O.D.) incubator for five days. The number of fungal colonies/100 seeds were determined.

The cockled seeds, water soaked (6 hr) or unsoaked were exposed to microwave irradiation for 0, 4, 8, 10, 12, 14... 30 sec. After exposure, both group of cockles were further soaked in water for 12 hr. The cockles were gently crushed to release the juveniles which were counted under a stereomicroscope.

Water soaked or unsoaked seeds of wheat cv. HD 2009 exposed to microwave irradiation for 0, 4, 8, 10, 12, 14... 30 sec were placed in Petri plates having two layers of sterilized blotter paper soaked with distilled water. Seed germination was determined after incubation of plates at 25 °C for five days.

Healthy seeds of wheat cv. HD 2009 together with the cockles soaked in water or unsoaked were exposed to microwave irradiation for 0, 4, 8, 12, 14, 16, 18 and 20 sec. The exposed healthy seeds along with cockles were sown at a depth of 2-3 cm in 25 cm clay pots (5 seeds + 5 cockles/pot) filled with autoclaved mixture of loam and farm manure (3:1). Five pots were maintained for each treatment. A week after sowing, seedlings were thinned to one per pot. Pots were watered weekly with tap water. Four months after sowing, plants were harvested and dry-weight of plant (excluding root and ears), numer of tillers and ears, number and weight of grains (including cockles)/plant and number of cockles (%) were determined. During the course of growth, plants were regularly examined for symptoms of nematode damage or irradiation injury.

The data on number of fungal colonies, seed germination and mortality of *A. tritici* juveniles were subjected to a single factor analysis of variance (ANOVA), whereas plant growth, yield etc. were analysed by two-factor ANOVA (Dospekhov, 1984). Data on seed germination were transformed by adding 0.5 and taking its underroot before the analysis of variance, (i.e. $\sqrt{x+0.5}$).

Results

More colonies developed in the Petri plates inoculated with healthy (normal) seeds of wheat than those with the cockles (Table I). Among the healthy seeds, water soaked seeds developed 27.7% more colonies than the unsoaked. Similarly, water soaked cockles also developed 20.3% colonies more. Effect of microwave irradiation on the mycoflora of healthy or cockled seeds whether soaked in water or not was found inhibitory and duration dependent (Table I). The inhibitory effect was greater on water soaked seeds than the unsoaked. The treatments from 8 and 10 sec onwards significantly decreased the number of fungal colonies on soaked healthy and cockled seeds, respectively. Significant decrease in the colonies from unsoaked healthy and cockled seeds occurred from 12 and 14 sec, respectively (Table I).

Exposure of cockles, soaked or unsoaked to microwave irradiation caused mortality of the juveniles of *A. tritici* (Table II). Juvenile mortality gradually increased with the increase in exposure duration. This effect was significant from 10 and 14 sec for soaked and unsoaked cockles, respectively. The mortality was much greater in soaked cockles than the unsoaked.

Microwave exposures caused varied effects on the germination of healthy wheat seeds (Table II). There was a marginal (insignificant) decrease in the germination of soaked or unsoaked seed upto 14 sec exposure duration. The seed germination from 16 sec onwards was inhibited gradually and significantly with both categories of seeds. From 22 and 24 sec onwards none of the soaked and unsoaked seeds, respectively, germinated (Table II).

Inoculated young plants (30-40 days old) grown from the seeds exposed to microwaves for 0-12 sec developed crinckling and rolling of

TABLE I - *Effect of microwave irradiation on mycoflora of healthy and cockled seeds of wheat.*

Irradiation exposure (sec)	Number of colonies/100 seeds			
	Healthy seeds		Cockled seeds	
	Soaked	Unsoaked	Soaked	Unsoaked
0	105	83	71	59
4	101	83	71	57
8	92*	81	68	57
10	79*	80	65*	57
12	53*	76*	65*	56
14	48*	74*	60*	54*
16	42*	73*	64*	49*
18	28*	71*	46*	49*
20	35*	68*	42*	47*
22	31*	68*	31*	46*
24	31*	59*	30*	41*
26	29*	54*	22*	37*
28	21*	46*	13*	36*
30	18*	46*	8*	32*
L.S.D. (P=0.05)	5.3	6.1	4.5	4.2
F=value (df=13)	92.5[a]	77.2[a]	89.4[a]	61.3[a]

Observations are based on 100 seeds; * Significantly different from the control at P=0.05; [a] Significant at P=0.05.

the leaves. Gall formation, however, occurred in all treatments (0-20 sec) and frequency was mild from 14 sec onward treatments. Infection of *A. tritici* caused significant increase in the number of tillers per plants. This increase, however, did not lead to any increase in ear formation, rather number of ears/plant was decreased by 4.2% (insignificant) compared with uninoculated plants (Table III). The infected plants exhibited significant decline in their dry weights. Treatments of seeds with microwave irradation led to varied effect on the plant growth of nematode inoculated and uninoculated wheat plants (Table III). Dry weight of uninoculated plants was decreased significantly due to 18 or 20 sec exposure. On inoculated plants, the microwave treatments gradually reduced the negative effect of nematode infection, as a result a significant decrease in dry weight of plants did not occur from 12 sec onwards and the difference between the dry weights of inoculated and uninoculated plants tend to decrease with an increase in exposure duration (Table III). These effects were more prominent in water soaked treatments. Response of number of tillers and ears was more or less similar to that of dry weight of plants (Table III). Greatest increase in the plant growth variables considered occurred at 14 or 16 sec exposure duration.

Infection of wheat plants with *A. tritici* significantly decreased the mean number and mean weight of grains/plant by 11 and 19% respectively, compared with uninoculated plants of both water soaked or unsoaked treatments (Table IV). Microwave treatments, however, suppressed the nematode pathogenesis, leading to a gradual decrease in the suppressive effect of nematode infection on the yield of wheat. As

a result, significant decline in the yield of inoculated plants of soaked and unsoaked categories did not occur from 12 and 16 sec, respectively. Microwave treatments itself, however, adversely affected the yield of uninoculated plants, especially at longer durations (Table IV). This effect was significant from 18 sec onwards for both soaked or unsoaked seeds.

A nematode inoculum level of approximately 22000 juveniles (5 cockles/plant, 4461 juveniles/cockle) caused the transmutation of more than 25% of normal grains into cockles or seed-galls (Table V). Number of cockles (soaked or unsoaked treatment) gradually decreased with an increase in exposure duration. This effect was significant from 8 sec (soaked) and 16 sec (unsoaked) onwards compared with unexposed treatment. Number of juveniles inside these cockles was also decreased with the increasing exposure duration, being significant from 12 and 18 sec onwards for soaked and unsoaked treatments, respectively (Table V).

Discussion

Juveniles of *A. tritici* fully or partially consume the endosperm of wheat (Southey, 1978), leaving less suitable substrate for the fungi. Probably for this reason, fewer fungal colonies developed on the cockles. Greater mycoflora of water soaked seeds may have developed due to higher water contents which stimulates the germination of fungal propagules (Mall *et al.*, 1986). Moisture also terminates dormant or resistant state of spores. Once the dormant state is

TABLE II - *Effect of exposure on cockles and healthy grains of wheat on mortality of juveniles of* Anguina tritici *and seed germination.*

Irradiation exposure (sec)	Mortality of juveniles (%)		Seed germination (%)	
	Soaked	Unsoaked	Soaked	Unsoaked
0	1.3	1.3	91.4 (9.6)	91.7 (9.6)
4	1.6	1.3	91.1 (9.6)	91.8 (9.6)
8	5.1	1.9	92.0 (9.6)	91.6 (9.6)
10	19.4*	4.5	90.1 (9.5)	92.1 (9.6)
12	24.5*	15.6*	90.3 (9.5)	91.8 (9.6)
14	39.8*	19.7*	88.4 (9.4)	85.6 (9.3)
16	43.2*	22.5*	80.0 (9.0)*	72.8 (8.6)*
18	45.7*	24.0*	64.1 (8.0)*	62.3 (7.9)*
20	51.5*	28.2*	13.4 (3.7)*	22.0 (4.7)*
22	54.8*	33.5*	0.0 (0.71)*	8.1 (2.9)*
24	60.7*	35.3*	0.0 (0.71)*	0.0 (0.71)*
26	66.0*	38.1*	0.0 (0.71)*	0.0 (0.71)*
28	69.5*	42.4*	0.0 (0.71)*	0.0 (0.71)*
30	76.1*	47.5*	0.0 (0.71)*	0.0 (0.71)*
L.S.D. (P=0.05)	5.2	4.7	0.43	0.40
L.S.D. (df=13)	177[a]	82[a]	16.5[a]	7.8[a]

* Significantly different from the control (10 sec) at P=0.05; [a] Significant at P=0.05; values in parenthesis are transformed values ($\sqrt{x+0.5}$).

TABLE III - *Effect of exposure of seeds of wheat to microwave irradiation on plant growth and ear formation of wheat.*

Treatment (sec)	Plant dry weight (g)		Tillers/plant		Ears/plant	
	Soaked	Unsoaked	Soaked	Unsoaked	Soaked	Unsoaked
Uninoculated	7.4	7.2	4.7	4.8	4.7	4.8
inoculated	6.3*	6.4*	5.0*	5.1*	4.5	4.7
Uninoculated	7.4	7.2	4.6	4.7	4.6	4.7
inoculated	6.2*	6.4*	4.9*	4.9	4.4	4.6
Uninoculated	7.4	7.2	4.7	4.6	4.7	4.6
inoculated	6.6*	6.4*	5.0*	4.8	4.5	4.4
Uninoculated	7.3	7.2	4.7	4.7	4.7	4.7
inoculated	6.9	6.6*	4.9	4.8	4.5	4.5
Uninoculated	7.3	7.2	4.7	4.7	4.7	4.7
inoculated	7.1	6.7*	4.8	4.9	4.6	4.5
Uninoculated	7.1	7.1	4.5	4.4*	4.5	4.4*
inoculated	6.9	6.7	4.7	4.5*	4.5	4.3*
Uninoculated	6.5*	7.0	4.1*	4.3*	4.1*	4.3*
inoculated	6.3	6.7	4.1	4.2*	4.0	4.1*
Uninoculated	6.2*	6.6*	3.4*	3.8*	3.3*	3.8*
inoculated	6.1	6.4	3.4*	3.8*	3.3	3.7*
L.S.D. (P=0.05)	0.53	0.47	0.28	0.30	0.25	0.26
F=value						
Nematode (df=1)	28.3[a]	21.8[a]	10.7[a]	5.3[a]	NS	NS
Microwave (df=7)	15.5[a]	8.2[a]	19.2[a]	17.1[a]	5.1[a]	9.5[a]
Interaction (df=7)	10.3[a]	5.9[a]	6.3[a]	NS	NS	NS

* Significantly different from the control at P=0.05; [a] Significant at P=0.05; NS=Not significant.

terminated, the fungal propagules become more sensitive to adverse factors, as exemplified by the greater decrease in colony count of soaked seeds than the unsoaked. The characteristics of the microwaves may also have contributed as they increase the temperature of moist material (Baranski and Czerski, 1976) as was the case with the mycoflora of soaked seeds.

A similar factor may also have been responsible for greater mortality of nematode juveniles in soaked cockles due to microwave irradiation. The quiescent stage of *A. tritici* juveniles inside the cockle is terminated by moisture (Southey, 1978). Thenafter they can not survive an exposure to 40 °C for 7 h (Kranjaic *et al.*, 1973). O'Bannon and Good (1971) reported that microwaves at 2450 MHz killed all the juveniles of *Meloidogyne incognita* in soil at a depth of 5 cm in 30 sec. In the present study 76% of juveniles inside the soaked cockles were killed by the microwave treatment for 30 sec.

Growth and yield reductions of uninoculated wheat plants grown from exposed seeds, especially for 18 or 20 sec indicates that microwaves induced some abnormality in the factor(s) that controls growth and yield. These effects persist-

ed in plants and occurred from seed germination till grain filling. The possibility of mutagenic changes can not be denied. Other irradiations, especially gamma rays is a well known physical mutagen and its exposure to wheat seeds may lead to growth and yield declines (Chowdhary and Nirmala, 1976).

Gradual increase in the growth and yield of nematode inoculated plants exposed to microwave irradiation from 4-16 sec was apparently due to adverse effects on the nematode pathogenesis. The effect gradually increased with the increase in exposure duration. Subsequently less cockles were formed, and the differences in plant growth or yield of inoculated and uninoculated plants became nonsignificant from 10 sec onwards.

The microwave treatments for 14 or 16 sec for soaked seeds was found to be the best with regard to improved yield and low nematode disease. These treatments did not cause any significant adverse effect on seed germination, plant growth or yield of wheat plants. The present results can be considered as an impor-

TABLE IV - *Effect of exposure of seeds of wheat to microwave irradiation on the yield.*

Treatment (sec)	Number of grains/plant		Weight of grains/plant	
	Soaked	Unsoaked	Soaked	Unsoaked
Uninoculated	112	117	7.59	7.51
inoculated	101*	103*	6.15*	6.02*
Uninoculated	109	114	7.61	7.31
inoculated	96*	101*	6.22*	5.74*
Uninoculated	115	118	7.53	7.49
inoculated	105*	106*	6.09*	6.14*
Uninoculated	114	109	7.57	7.25
inoculated	108	96*	6.90	6.29*
Uninoculated	112	108	7.63	7.21
inoculated	111	101	7.28	6.67*
Uninoculated	105	107	7.57	7.19
inoculated	102	103	7.35	6.75*
Uninoculated	94*	93*	7.08*	6.12*
inoculated	93	88	6.91	5.93
Uninoculated	81*	82*	6.40*	5.52*
inoculated	79	78	6.27	5.37
L.S.D. (P=0.05)	9.1	10.4	0.49	0.52
F=value				
Nematode (df=1)	19.7[a]	13.5[a]	25.5[a]	35.2[a]
Microwave (df=7)	12.2[a]	12.1[a]	NS	13.6[a]
Interaction (df=7)	NS	17.4[a]	NS	9.1[a]

* Significantly different from the control at P=0.05; [a] Significant at P=0.05; NS=Not significant.

TABLE V - *Effect of exposure of seeds of wheat to microwave irradiation on the ear-cockle disease.*

Treatment (sec)	Cockles %		Number of larvae/cockle	
	Soaked	Unsoaked	Soaked	Unsoaked
0	25.7	27.4	4351	4572
4	26.1	28.3	4396	4705
8	21.7*	27.0	4401	4611
12	18.1*	26.3	4817*	4507
14	10.9*	25.5	3685*	4338
16	7.3*	21.7*	2915*	4275
18	5.1*	15.3*	2107*	3960*
20	1.7*	12.9*	1374*	2905*
L.S.D. (P=0.05)	2.1	2.40	391	426
F=value	81.6[a]	42.7[a]	71.3[a]	19.4[a]

* Significantly different from the control at P=0.05; [a] Significantly at P=0.05.

tant physical method for management of ear-cockle disease. In India and other developing countries, the growers have become concerned with seed-borne diseases and microwave treatment may serve as an effective and handy tool for seed disinfestation, if the technique is explored properly.

Acknowledgements. The authors are grateful to Prof. M. Shamim Jairajpuri, the then Director of Institute of Agriculture, A.M.U., for providing necessary facilities to carry out the study.

Literature cited

BARANSKI S. and CZERSKI P., 1976. *Biological Effects of Microwave.* Dowden, Hutchinson and Ross, Stroudsburg, pp. 63-70.

BROWN R. H. and KERRY B. R., 1987. *Principles and Practices of Nematode Control in Crops.* Academic Press, New York, U.S.A., 447 pp.

CHOWDHRY J. B. and NIRMALA, 1976. Effects of Gamma rays on seedling height and chromosome aberration in *Triticale. Acta Bot. Indica, 4*: 126-130.

DOSPEKHOV B. A., 1984. *Field Experimentation.* Mir Publishers, Moscow, Russia, 352 pp.

KHAN M. R. and ATHAR M., 1996. Response of wheat cultivars to different inoculum levels of *Anguina tritici. Nematol. medit., 24*: 269-272.

KRANJAIC D., KRANJAIC S. and OTASECIS R., 1973. Longevity of wheat nematode at different temperature. *Zastita Bilja, 24*: 67-73.

MALL O. P., PATERIA H. M. and CHAUHAN S. K., 1986. Mycoflora and alfatoxin in wet harvested sorghum. *Indian Phytopathol., 39*: 409-413.

O'BANNON J. H. and GOOD J. M., 1971. Application of microwave energy to control nematodes in soil. *J. Nematol., 3*: 93-94.

SOUTHEY J. F., 1978. *Plant Nematology.* Her Majesty's Stationary Office, London, U.K. 440 pp.

SWARUP G. and SOSA-MOSS C., 1990. Nematode parasites of cereals, pp. 109-136. *In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (Luc M., Sikora R. A. and Bridge J. Eds). CAB International, Wallingford, UK.

Accepted for publication on 3 May 1999.