

**Microwave radiation for the management of red
flour beetle, *Tribolium castaneum* (Herbst)
(Coleoptera: Tenebrionidae)**

By
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(2019-11-141)



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KERALA, INDIA

2022

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Department of Agricultural Entomology

**COLLEGE OF AGRICULTURE
VELLANIKKARA, THRISSUR- 680656
KERALA, INDIA**

2022

DECLARATION

I, Sikha Raju (2019-11-141) hereby declare that the thesis entitled “**Microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)**” is a bonafide record of research done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 11/04/2022



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Certified that this thesis entitled “**Microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)**” is a record of research work done independently by **Ms. Sikha Raju (2019-11-141)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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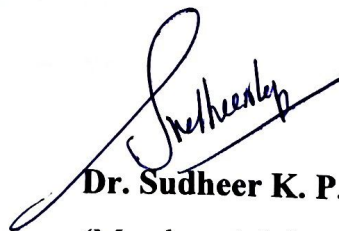
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CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-16
3	MATERIALS AND METHODS	17-23
4	RESULTS	24-68
5	DISCUSSION	69-80
6	SUMMARY	81-82
	REFERENCES	i-x
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	HPLC parameters for the analysis of vitamin B	23
2	Effect of power of microwave radiation on two-day old eggs of <i>Tribolium castaneum</i>	25
3	Effect of flour bed thickness on two-day old eggs of <i>Tribolium castaneum</i>	25
4	Effect of exposure time on two-day old eggs of <i>Tribolium castaneum</i>	27
5	Interaction effect of power and thickness on two-day-old eggs of <i>Tribolium castaneum</i>	27
6	Interaction effect of power and time on two-day-old eggs of <i>Tribolium castaneum</i>	30
7	Interaction effect of thickness and time on two-day-old eggs of <i>Tribolium castaneum</i>	30
8	Interaction effect of power, thickness and time on two-day-old eggs of <i>Tribolium castaneum</i>	35-36
9	Effect of power of microwave radiation on 20±2 day old grubs of <i>Tribolium castaneum</i>	36
10	Effect of flour bed thickness on 20±2 day old grubs of <i>Tribolium castaneum</i>	37
11	Effect of microwave exposure time on 20±2-day old grubs of <i>Tribolium castaneum</i>	37
12	Interaction effect of power and thickness on 20±2-day old grubs of <i>Tribolium castaneum</i>	40
13	Interaction effect of power and time on 20±2-day old grubs of <i>Tribolium castaneum</i>	40
14	Interaction effect of power, thickness and time on 20±2-day old grubs of <i>Tribolium castaneum</i>	44-45

15	Effect of power of microwave radiation on pupae of <i>Tribolium castaneum</i>	45
16	Effect of flour bed thickness on pupae of <i>Tribolium castaneum</i>	46
17	Effect of microwave exposure time on pupae of <i>Tribolium castaneum</i>	46
18	Interaction effect of power and thickness on pupae of <i>Tribolium castaneum</i>	48
19	Interaction effect of power and time on pupae of <i>Tribolium castaneum</i>	48
20	Interaction effect of thickness and time on pupae of <i>Tribolium castaneum</i>	50
21	Interaction effect of power, thickness and time on pupae of <i>Tribolium castaneum</i>	54-55
22	Effect of power of microwave radiation on 17±2 day old adults of <i>Tribolium castaneum</i>	55
23	Effect of flour bed thickness on 17±2 day old adults of <i>Tribolium castaneum</i>	56
24	Effect of microwave exposure time on 17±2 day old adults of <i>Tribolium castaneum</i>	56
25	Interaction effect of power and thickness on 17±2 day old adults of <i>Tribolium castaneum</i>	59
26	Interaction effect of power and time on 17±2 day old adults of <i>Tribolium castaneum</i>	59
27	Interaction effect of thickness and time on 17±2 day old adults of <i>Tribolium castaneum</i>	61
28	Interaction effect of power, thickness and time on 17±2 day old adults of <i>Tribolium castaneum</i>	66-67
29	Effect of microwave radiation on moisture, gluten and vitamin B content of wheat flour	67
30	Effect of microwave radiation on colour of wheat flour	68

LIST OF FIGURES

Figure No.	Title	Page No.
1	Susceptibility of various stages <i>Tribolium castaneum</i> to microwave radiation	71

LIST OF PLATES

Plate No.	Title	After page
1	Test insects culture	18
2	Microwave oven	18
3	Samples prepared for irradiation	18
4	B.S.S. 60 sieve	18
5	B.S.S 18 sieve	18
6	Two day old eggs	20
7	20±2 day old grub	20
8	Pupa	20
9	17±2 day old adult	20
10	Hot air oven	23
11	Hunter MiniScan	23
12	Normal grub and malformed grub	68
13	Normal and malformed pupa (a) dorsal view (b) ventral view	68
14	Normal and malformed adult (a) dorsal view (b) ventral view	68

Introduction

1. INTRODUCTION

Cereals hold a unique and supreme position in daily diet among food grains as they are a good source of carbohydrate (65-75 per cent), protein (7-12 per cent), lipids, vitamins, minerals and dietary fibre (Baniwal *et al.*, 2021). India is the second-largest producer of cereals in the world. Among cereals, wheat ranks second in production and is rich in carbohydrates (78.10 per cent), protein (14.70 per cent), fat (2.10 per cent), minerals (Zn, Fe), vitamin B and dietary fibre. Gluten is the major protein in wheat, providing stickiness to the dough. In addition, wheat bran and germ contain higher amounts of carotenoids and antioxidants (Kumar *et al.*, 2011).

Post-harvest damage by insects is one of the major constraints associated with whole and processed grain storage, adversely affecting physical and nutritional properties. Further, contamination with insect's body and their products followed by mould growth will render the product unfit for consumption, and thus diminish the market value (Rees, 2004). In India, post-harvest loss accounts for 10 per cent of total food grain production, in which the share of loss by insects alone is 2.00 to 4.20 per cent (IGMRI, 2019).

Red flour beetle, *Tribolium castaneum*, is a cosmopolitan stored grain pest belonging to the family Tenebrionidae. Tenebrionidae is a large and diverse family of tiny beetles with over 10,000 species, almost 100 of which are pests on a variety of stored goods (Rees, 1996). *Tribolium castaneum* has been reported on wheat and wheat flour, cereal-based food stuffs, and even dry fruits (Devi and Devi, 2015). The beetle spoils the wheat flour with certain chemical excretions, body fragments, dead insects and renders an off odour. The study on nutritional loss in wheat due to *T. castaneum* found a positive correlation in protein and fat composition, while carbohydrate content was unaffected (Wakil *et al.*, 2003).

Chemical control is the widely adopted practice for the management of red flour beetle. Fumigation is an economical and effective management option. However, the residual effect of chemicals, pest resurgence, resistance development in insects, and adverse environmental impact are critical sides of chemical control (Abdullahi *et al.*, 2019). Resistance development is a major concern in the storage ecosystem due

to the continuous selection pressure imposed by insecticides (Boyer *et al.*, 2012). Anusree *et al.* (2019) reported above 10.95 fold malathion resistance in *T. castaneum* collected from different FCI godowns of Kerala over the susceptible strain. Such studies extend constant demand for novel, eco-friendly as well as economic strategies for storage pest management.

Physical, cultural and biological methods are promising bio rational management measures gaining attention worldwide. Physical control measures include temperature manipulation, irradiation, controlled and modified atmospheres, humidity control, desiccation and so on (Phillips and Throne, 2010).

Disinfestation of storage pests using non-ionizing microwave radiation is an effective eco-friendly strategy with no residual effect on food, adverse effects on human beings, and fewer chances of resistance development in insects. During microwave irradiation, alternating electromagnetic field energy is transformed to thermal energy due to the dielectric heating of polar molecules in materials. As the materials absorb microwave energy, the dipolar molecules, mainly water, try to align in the alternating electromagnetic field. Thus friction is generated between the molecules, which converts the electromagnetic energy to heat, and the process is called dielectric heating (Vadivambal *et al.*, 2007). In addition, microwave heating adversely affects the physiological processes in insects infesting stored products, affecting their survival and reproductive capacity (Webber *et al.*, 1980).

Against this backdrop, the current study proposed to investigate the feasibility of “microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)” with the objectives

1. To study the effectiveness of household microwave system for the management of red flour beetle, *Tribolium castaneum*
2. Study the effect of microwave radiation on nutritional factors of wheat flour

Review of literature

2. REVIEW OF LITERATURE

Humans have consumed cereals for thousands of years. Grains and their products are rich in energy, carbohydrate, protein, fibre, and various micronutrients. The attack of storage pests, which has a negative impact on grain quality, is one of the most important threats to their storage. Chemical fumigation is the widely adopted management method but, resistance development, residual effect of chemicals in grains and environmental concerns demanded a better management strategy. Physical, cultural and biological methods are promising biorational management measures gaining attention worldwide. Starting from sanitation measures which are considered as the first line of defense in pest management, physical control measures include temperature manipulation, irradiation, controlled and modified atmospheres, humidity control and desiccation and so on (Phillips and Throne, 2010).

Irradiation is the process of applying electromagnetic radiation (ionizing or non-ionizing) of specific wavelengths and energy to a commodity in order to suppress pests. Ashraf *et al.* (2021) studied the efficacy of ionizing gamma radiation in management of all developmental stages of pulse beetle, *Callosobruchus maculatus* without compromising the grain quality. According to recent studies, microwave heating is also considered as a feasible solution in alleviating storage pest infestations without having a negative impact on the environment.

Previous works on microwave radiation for the management of stored grain pests are reviewed and presented in this chapter. In addition, effect of irradiation on different life stages of insects is consolidated and reviewed.

2.1 EFFECT OF MICROWAVE RADIATION ON EGGS OF STORED GRAIN PESTS

Shayesteh and Barthakur (1996) studied the effect of microwave radiation on the eggs (12-24 h old) of *Tribolium confusum* at different levels of treatments *viz.*, 75W, 100W and 150W by varying the exposure time. Eggs were more susceptible

when compared to other life stages. Irradiation at 75W and 100W power levels intermittently for 20 and 10 minutes, respectively, resulted in complete mortality.

Zhao *et al.* (2007) reported that microwave irradiation of eggs and adults of rice weevil at low power and longer exposure time ensured 100 per cent mortality without compromising the quality of grain.

Vadivambal *et al.* (2008) studied the mortality of eggs, larvae, pupae, and adults of *Tribolium castaneum* at various power levels, irradiation periods, and moisture content of barley. The mortality of *T. castaneum* eggs treated with microwaves at 200, 300, and 400 W were 41, 94, and 100 per cent, respectively, at 28-s exposure time, and 91 and 100 per cent, respectively, at 200 and 300 W, at 56-s exposure time.

Eggs of Indian meal moth in different age groups (1-4day old eggs) were irradiated and kept under cold storage for 24 and 48 h to study the combined impact of microwave irradiation and cold storage treatment (Nasab *et al.*, 2010). The results showed that one and two-day old eggs were least susceptible to treatment since the embryonic development was low during the initial two days.

Azizoglu *et al.* (2011) studied the ovicidal activity of microwave radiation on Mediterranean flour moth, *Ephesia kuehniella*, by exposing the eggs to four different doses (150, 360, 430 and 600 W) for different periods. There was a proportional change in the mortality of eggs and irradiation doses, *ie.*, as the radiation dose increased, the mortality of eggs increased due to decreased embryonic development of eggs. The median lethal time (LT₅₀) values at each dose showed an inverse trend, *ie.*, at a lower dose of 150 W, LT₅₀ was 103.34 s. By increasing the dose to 600 W, LT₅₀ decreased to 2.61 s.

Microwave heating has an immense impact in controlling all developmental stages of rice weevil, *Sitophilus oryzae* (Franco *et al.*, 2013). Exposure of eggs to 240 W for shorter periods was non-detrimental. But for the same irradiation dose, the adult emergence was nil when the exposure time increased to 130 s.

The study conducted by Purohit *et al.* (2013) regarding the influence of different levels of microwave radiation (200, 300, 400 W) on eggs of *Callosobruchus maculatus* revealed that egg development completely ceased at 400W.

The microwave power adversely affected the egg hatchability and progeny count of *C. maculatus* infesting cowpea. The hatchability of eggs was 85.5 per cent at a dose-time combination of 200 W, 20 s, which dropped to zero per cent at a treatment combination of 600 W, 30 s. The reduction in progeny production was 5.5 per cent at 200 W, which changed to 100 per cent at 600 W (Shoughy and Elzun, 2014).

Khalaf *et al.* (2015) demonstrated the feasibility of microwave technique over chemical treatment to control different biological stages of *Ephestia cautella* in dates. At 600 W and exposure for 20 s resulted in the highest mortality of eggs. However, the time taken to reach complete mortality decreased as the irradiation dose increased. Thus at 800 and 1000 W power levels, 100 per cent mortality occurred after 14 and 12 s of exposure, respectively.

Tayeb *et al.* (2018b) standardised the duration of microwave treatment at 900 W to manage immature stages of *Callosobruchus chinensis* on faba beans. The adult emergence from treated eggs decreased significantly as the duration of treatment increased. For example, adult emergence was 65.99 per cent after 5 s of exposure, while it was zero after 30 s.

Minj *et al.* (2021) reported cent per cent mortality of *Lasioderma serricorne* eggs in microwave irradiated turmeric powder at 550 W and 650 W for 10 and 8 minutes, respectively, without compromising the colour of turmeric powder.

Microwave disinfestation of all biological stages of *Caryedon serratus* and *Corcyra cephalonica* on groundnut and sesame seeds were studied by Patil *et al.* (2021). There was no larval emergence after treating eggs of *C. serratus* and *C. cephalonica* at 480 W for 60 s and 600 W for 30 s, respectively.

2.2 EFFECT OF MICROWAVE RADIATION ON LARVAE OF STORED GRAIN PESTS

Shayesteh and Barthakur (1996) concluded that larvae of *T. confusum* were more resistant to microwave radiation. The mortality of larvae was 100 per cent when treated at 100 and 150 W for 20 and 10 minutes, respectively.

Vadivambal *et al.* (2007) irradiated the larvae of *T. castaneum* with different doses of microwave radiation ranging from 250 to 500 W for various periods. They observed complete inhibition of larval development at 500 W. Later, Vadivambal *et al.* (2008) calculated that *T. castaneum* larvae would die at 300 or 400 W after being exposed for 56 s.

Date moth, *Ectomyelois ceratoniae*, a serious pest of both field and storage in dates, was effectively controlled by physical means using microwave radiation. The most heat tolerant fourth or fifth instar larvae were selected to artificially infest on soft and dry dates and exposed to 1000 W power for 55, 60, 70 and 90 s. Complete mortality of larvae was reported in soft dates at all exposure periods. In contrast, in dry dates, cent per cent mortality could not be achieved as larval death temperature was not attained (Zouba *et al.*, 2009).

Vadivambal *et al.* (2010) reported complete mortality of larval stages of *Sitophilus zeamais*, *T. castaneum* and *Plodia interpunctella* at 600 W for 14 s irradiation time. *Plodia interpunctella* larvae were more susceptible than *T. castaneum*.

El-Naggar and Mikhael (2011) analysed the mortality of *T. confusum*, *L. serricornis* and *C. cephalonica* upon microwave treatment. There was no mortality of *T. confusum* and *L. serricornis* larvae at 40°C, and 10 s exposure time and mortality of *C. cephalonica* larvae was only 11.3 per cent. The mortality of *T. confusum* larvae at 50°C increased from 20.33 to 100 per cent, as the exposure time increased from 10 to 50 s. At 40, 45, 50, and 55°C, mortality for *L. serricornis* larvae were 31.7, 37.7, 66.3, and 100 per cent, respectively, for a 30 second exposure time. The most susceptible *C. cephalonica* larvae died at 50°C at a lower exposure time of 20 s.

Irradiation at 808 W for 80 s prevented the larval development and adult survival of *Callosobruchus* sp. (Pande *et al.*, 2012). Hajmohammadi *et al.* (2013) reported a significant decline in the survival of the Indian meal moth, *Plodia interpunctella* in pistachio after microwave treatment. The per cent mortality of fifth instar larvae was 39.11 at 20 s and as the exposure time increased to 50 s, there was complete mortality of the larvae.

Pandir and Guven (2014) examined the influence of microwave treatment on the mortality of *Ephesia kuehniella* larvae. They found that exposure to a low dose (70 W) for 50 s leads to the mortality of one-two day old larvae. At a high dose of 600 W, complete mortality was reported from 5 s exposure time onwards. An exposure for 50 s resulted in damage to DNA. LT₅₀ and LT₉₉ values at 600 W were 2.304 and 4.62 s, respectively.

The larval mortality of *E. cautella* at 600, 800 and 1000 W power levels and 10 s exposure time was 90, 90 and 93.3 per cent, respectively. Doubling the treatment time resulted in complete mortality at 1000 W and a high mortality rate of 96.70 per cent at 600 and 800 W (Khalaf *et al.* 2015).

Meenatchi *et al.* (2015) conducted disinfestation studies of *Sitophilus zeamais* and *T. castaneum* using a domestic microwave oven at four power levels (270, 360, 450 and 540W) and three exposure times (20, 25 and 30 s). Larval mortality of both test insects was 100 per cent at a treatment combination of 540 W and 20 s.

Irradiation experiment performed on stored product mite, *Tyrophagus putrescentiae* infested wheat flour indicated the influence of moisture content on mortality of its larvae (Bakr, 2017). The mortality of larvae was high on flour with higher moisture content and larval mortality was 100 per cent after application of radiation at 110 W and 330 W for a period of 300 and 60 s, respectively.

According to Barbosa *et al.* (2017), microwave radiation at 240 W dose and 120 and 150 s exposure period resulted in complete suppression of adult emergence from *C. maculatus* larvae. In addition, there was a significant reduction in pest damage and an increase in egg to adult period.

Sadeghi *et al.* (2018) standardised the microwave power and exposure time for the complete disinfestation of *E. kuehniella* larvae (fifth instar) in dried figs and raisins. Larvae failed to pupate when exposed to 900 W for 50 s.

Tayeb *et al.* (2018a) compared the sensitivity of larvae of *T. castaneum* in different age groups (1-7, 7-14 and 14-21 days) to irradiation. One to seven-day old larvae were highly sensitive at 800 W with a short exposure time of 5 s.

Tayeb *et al.* (2018b) calculated the per cent reduction in adult emergence from microwave-irradiated *C. chinensis* larvae at six time levels. Adult emergence completely reduced when the duration of exposure was 25 and 30 s. They also discovered that eggs and larvae are the most vulnerable stages to irradiation.

2.3 EFFECT OF MICROWAVE RADIATION ON PUPAE OF STORED GRAIN PESTS

Watters (1976) evaluated the impact of microwave radiation on the survival of *T. confusum* as a function of exposure time and moisture content of wheat. Most pupae at the top layer of wheat emerged to adults, whereas those at the middle and bottom layers were found dead. Non-uniform temperature distribution associated with microwave heating is the reason for the variation in mortality.

Shayesteh and Barthakur (1996) reported that intermittent or continuous irradiation of 12-24 h old pupae of *T. confusum* at 100W could inhibit adult emergence when exposed for 10 and 20 minutes, respectively.

Halverson *et al.* (2003) conducted a study to analyse the most vulnerable developmental stage towards microwave radiation among three different test insects like *S. granarius*, *T. castaneum* and *R. dominica*. The developmental stages selected for the study were egg, larvae and pupae. The study showed that pupae of *R. dominica* were most vulnerable to microwave energy.

The pupal death rate of *T. castaneum* increased from 43 to 76 per cent as the power level raised from 250 to 400 W at 28 s treatment time. There was total mortality when irradiated at 500 W. Raising the exposure time to 56 s, resulted in

complete pupal mortality at a lower radiation dose of 400 W (Vadivambal *et al.*, 2007).

The response of *T. castaneum* pupae varied significantly with the degree and duration of microwave radiation (Vadivambal *et al.*, 2008). The pupal mortality was 19, 41, and 100 per cent at 200, 300, and 400 W, respectively, for 28 s exposure. Mortality was significantly higher at higher power levels and raising the exposure time to 56 seconds increased the mortality to 63, 94, and 100 per cent at the corresponding power levels.

Complete mortality of all the life stages of *C. maculatus*, except pupae, was achieved at 600 W. Pupae, being the least susceptible stage, required a higher dose (800 W) for adult emergence to be zero (Shoughy and Elzun, 2014).

According to Khalaf *et al.* (2015), a difference in response of male and female pupae of *E. cautella* to microwave treatment was evident. Female pupae were more susceptible than males due to the variation in the liquid content in their bodies. At 800 W and 18 s exposure time complete mortality of females was recorded, whereas for males, the exposure time was raised to 20 s to attain complete mortality.

According to Tayeb *et al.* (2018a), 1-day old male pupae of *T. castaneum* were more susceptible to radiation than female pupae. Tayeb *et al.* (2018b) reported that pupae of *C. chinensis* were the most tolerant immature stage to microwaves. As a result, the fatal effect was much smaller at shorter exposure times.

Patil *et al.* (2020) conducted experiments to determine the effect of microwave radiation to *T. castaneum* infested almond samples. The complete death of the most tolerant pupal stage was obtained at a treatment combination of 600 W and 60 s, whereas larvae and adults died at a lower dose of 480 W.

Microwave-assisted disinfestation of all life stages of *C. maculatus* was conducted on black gram by Tiwari *et al.* (2021). Pupae were least vulnerable to radiation with a higher LT_{99.99} of 8.87 min at 365 W radiation dose. LT_{99.99} of egg, larvae and adults were 5.33, 8.28 and 8.86 min, respectively.

Irradiation at 600 W for 60 s completely eradicated the pupal stages of *T. castaneum* and *C. serratus* on peanuts. Whereas a low dose of 360 W for 60 s was enough to control *C. cephalonica* pupae on sesame (Patil *et al.*, 2021).

2.4 EFFECT OF MICROWAVE RADIATION ON ADULTS OF STORED GRAIN PESTS

Vadivambal *et al.* (2008) discovered that a higher dose and duration of radiation was required to achieve complete mortality of adults of *T. castaneum*, as they are the least vulnerable life stage.

Vadivambal *et al.* (2009) used a pilot-scale industrial microwave dryer to conduct disinfestation studies of three stored-grain pests, namely *T. castaneum*, *Cryptolestes ferrugineus* and *Sitophilus granarius*. They treated infested grain samples of wheat, barley at four irradiation doses (200, 300, 400, and 500 W) and two exposure times (28 and 56 s). In wheat and barley, two dose-time combinations *ie.*, 500 W, 28 s and 400 W, 56 s resulted in the death of adults of all the three insects. The dose-time combination for complete mortality of adults of *T. castaneum* and *S. granarius* in rye was 300 W and 56 s, whereas for *C. ferrugineus*, it required 500 W, 28 s and at 400 W, 56 s to achieve complete mortality.

Gasemzadeh *et al.* (2010) recommended that a combination treatment of microwave radiation and cold storage effectively control adult stages of *Tribolium castaneum* and *Sitophilus oryzae*.

Microwave heating at a temperature level of 40°C for 50 s instantaneously killed adults of *C. cephalonica* whereas, to accomplish complete mortality of *T. confusum* and *L. serricorne*, temperature level needs to be raised to 50°C (El-Naggar and Mikhael, 2011).

Valizadegan *et al.*, 2011 investigated the effect of microwave radiation with cold storage on adults of saw-toothed grain beetle, *Oryzaephilus surinamensis* and cigarette beetle, *L. serricorne*. They subjected the insects to radiation doses of 100, 200, 300 and 400 W for 3, 6, 9 and 12 min duration and maintained the insects at cold

storage conditions ($4 \pm 1^\circ\text{C}$) for 24, 48, and 72 h, after irradiation. The adults of both the beetles failed to survive at 400W irradiation dose for 12 minutes exposure time and cold storage condition treatment for 72h.

Singh *et al.*, (2012) ascertained the mortality of adults of *C. chinensis* exposed to microwave radiation (2450 MHz) as a function of exposure time and per cent power level. The adults reared on chickpea, administered with power levels such as 700, 560, 420, 240 and 140 W for 100, 160, 200, 240 and 300 s, respectively, resulted in the immediate death of the beetles. Furthermore, during microwave exposure, insects moved towards the top layer of grain.

According to Franco *et al.* (2013), there was instantaneous death of adults of rice weevil when irradiated at 240 W power levels for 160 s. The longevity and reproductive fitness of adults significantly reduced with the increase in exposure time.

Complete control of adults of *T. castaneum* and *O. surinamensis* infested on stored dates was obtained at an irradiation dose of 800 W, exposure for 40 s (Manickavasagan *et al.*, 2013). Purohit *et al.* (2013) reported that all the adults of *C. maculatus* failed to survive at 400 W irradiation dose for a 28 s exposure period.

Echereobia *et al.* (2014) studied the efficacy of microwave heating on post-harvest control of cowpea bruchid. After treating adults (1-2 days old) at 100 W for varying exposure periods (0, 2, 4, 6, 8 and 10 minutes), mortality was recorded at 24, 48, 72 and 96 h intervals. The highest mortality was at 8 min exposure. An exposure to microwave irradiation for 10 minutes had the least weevil perforation index in cowpea seeds.

Khalaf *et al.* (2015) observed the effect of irradiation on longevity of male and female adults of *E. cautella*. The longevity of emerged male and female adults in control was 15.2 and 17.6 days, respectively. At 600 W, 6 s treatment, the longevity reduced to 4.3 days in males and 5.3 days in females.

Jian *et al.* (2015) evaluated the expelling effect of microwaves on *C. ferrugineus* from wheat samples. Microwave treatment expelled all the introduced

adults, and progeny production decreased by 75 per cent but didn't kill the insects completely.

Ahmady *et al.* (2016) studied the susceptibility of *T. confusum* and *C. maculatus* to microwave heating (400W) at different time duration (5, 10, 15, 20, 25 s). All *T. confusum* adults died when irradiated for 25 s, whereas mortality was 98.8% in *C. maculatus* for the same exposure time. Increasing the treatment time resulted in a drastic decline in fecundity and progeny emergence.

Saboori *et al.* (2017) compared the susceptibility of both sexes of *C. maculatus* upon radiation treatment. The findings showed that as the exposure period and or the power of radiation increased, the mortality rates of male and female individuals increased. Maximum mortality was achieved in 960 s and 80 s at 90 W and 900 W, respectively, implying that as microwave power levels increased, the time elapsed to achieve maximum lethal effect decreased. In addition, they observed a high tolerance level of females to irradiation rather than males.

Tayeb *et al.* (2018a) concluded that adults of *T. castaneum* (2 week-old) were least susceptible to irradiation than pupae, which was proved through the median lethal time values. LT₅₀ of adults (8.951 ± 0.114 s) was higher than pupae (4.015 ± 0.230 s).

Keszthelyi *et al.* (2019) recorded a remarkable change in progeny production in granary weevil, *S. granarius*, upon microwave treatment. Progeny production declined to half of the normal rate at 100 W, and it further reduced to zero at 300 W.

Mohapatra *et al.* (2019) disinfested green gram and chickpea infested with adults of *C. maculatus*. A temperature of 60°C and power level of 2900 W for 6 minutes resulted in complete death of adults exposed.

The research conducted on the potential of microwaves in pest control of *T. castaneum* and *Trogoderma granarium* indicated the adults to be more susceptible than the larval stage. However, there was no significant change in enzyme activity (Abotaleb *et al.*, 2021).

The effect of microwave radiation on the management of stored pest and progeny development was studied on *S. oryzae* and *T. castaneum* by Draz *et al.* (2021). At 1200 W and 5 s exposure time, there was no change in adult mortality. But, as the period increased to 25 s, the mortality increased significantly. The median lethal time (LT₅₀) of both insects were 13 s and 14 s, respectively, and progeny emergence was also negatively affected by radiation.

2.5 EFFECT OF THICKNESS OF SAMPLE IN DISINFESTATION USING MICROWAVE RADIATION

During disinfestation studies of *T. confusum*, *S. granarius*, and *C. ferrugineus*, Hamid *et al.* (1969) discovered that the samples should be treated in thin layers of less than 0.1 m to achieve satisfactory mortality rates.

Mohapatra *et al.* (2014) conducted a study to discover the efficacy of microwave radiation in the disinfestation of cowpea bruchid by varying the thickness of the grain bed from single layer to 2 cm. The time for mortality of adults increased as the grain bed depth increased and there was no mortality in any of the samples within 30 seconds of exposure. Complete mortality of adults occurred in 50, 60, 90, 110, and 120 s for single layer, 0.5, 1, 1.5, and 2 cm thickness, respectively.

The penetration depth of microwave radiation is inversely proportional to the frequency of radiation. At a lower frequency of 915MHz, depth ranged between 7.4 to 8.6 cm, and at 2450 MHz, it decreased to 4.3 to 5.4 cm. So for uniform heating and complete disinfestation, treatment of cereal samples at thin layers are recommended (Torrealba-Melendez *et al.*, 2015)

2.6 EFFECT OF MICROWAVE ON PHYSICAL AND NUTRITIONAL QUALITIES OF STORED GRAIN

Campana *et al.* (1993) reported a significant reduction in the wet gluten content of wheat flour with an increase in exposure time upon microwave drying. Walde *et al.* (2002) also reached a similar conclusion regarding gluten content upon microwave heating. Gluten provides elasticity and stretchability to the dough by

binding the wheat particles together. After the treatment, an alteration in these functional properties occurred due to the reduction in gluten content.

A reduction in moisture content by 1.4 per cent was observed on microwave heating of stored dates, whereas there was no variation in the surface colour (Manickavasagan *et al.*, 2013).

Meenatchi *et al.*, (2015) reported a moisture loss of only one per cent after disinfestation of wheat samples at microwave power levels of 360 and 450 W. However, at a higher power level of 540 W, moisture loss was estimated to be four per cent.

Microwave heating, even though ensured the disinfestation of storage pests, it negatively affected qualitative parameters of stored product. Increasing the power level and exposure duration, the moisture content, total protein, and total carbohydrate in treated wheat grains, wheat flour and cowpea grains declined (El-Raheem and Said, 2016).

Qu *et al.* (2017) compared gluten content in wheat before and after microwave heating at 700 W power for varying time periods (10, 20, 30, 40, 50, 60 s). There was no considerable variation when wheat samples were exposed for less than or equal to 20 s. Microwave heating for longer periods *ie.*, ≥ 30 s, resulted in the rise in temperature to ≥ 68 °C, denaturing gluten and damaged its structure.

Variation in organoleptic properties, including the colour of microwave treated dried fig and raisins, was studied by Sadeghi *et al.* (2018) and reported a substantial change in colour with a high degree of microwave radiation and exposure time.

Mohapatra *et al.* (2019) examined the effect of hot air-assisted microwave heating on green gram and chickpea colour using CIE lab scan. In green gram, the greenness and lightness in the treated sample decreased, whereas, in the case of chickpea, the redness increased. They concluded that the colour change might be due to rise in temperature during heating, which resulted in the browning of samples.

Hassan *et al.* (2021) concluded that microwave heating does not produce any adverse effect on the nutritional qualities and colour of corn grain.

2.7 EFFECT OF MICROWAVE RADIATION ON THE PHYSIOLOGY OF INSECT

Lu *et al.* (2010) noticed a reduction in alkaline phosphatase activity in *Tribolium castaneum* as the power of low intensity microwave radiation (LIMR) treatment increased. This justified the decreasing heat tolerance in beetles with the increasing power levels. They also reported DNA damage due to irradiation.

Lu *et al.* (2011) reported the adverse impact of microwave radiation on the cellular ultrastructure of *T. castaneum*. Due to irradiation, the layers of cuticle became thin with ambiguous boundaries. In mesodermal cells, damage of mitochondria and Golgi bodies was noticed and vacuoles get emptied due to loss of water.

Azizoglu *et al.* (2011) stated that proliferation and development of embryonic cells are slower at the egg stage compared to later developmental stages of *Ephestia kuehniella*. This made the eggs to be the most vulnerable stage towards radiation treatment.

Panagopoulos (2012) studied the effect of microwave radiation on the ovarian development of virgin females of *Drosophila melanogaster*. A significant reduction in size of ovaries was noticed in irradiated females as compared to control due to DNA damage in egg chamber cells.

The study in microwave treated *Ephestia kuehniella* larvae, at a high-power level of 300 and 600 W, revealed a decrease in antioxidant enzyme activities like SOD, CAT, GPx and increase in MDA level, a toxic product of polyunsaturated fatty acid peroxidation. At a higher dose of 600 W, DNA damage was also noted (Pandir and Guven, 2014).

Tungjitwitayakul *et al.* (2016) conducted a study on the response of three heat shock proteins (*hsps*) upon microwave irradiation of maize weevil. The expression of *hsps* was enhanced in all stages during the irradiation with exposure time up to median

lethal time. The activity of *hsps* was highest in adults, which conferred their higher tolerance to radiation treatments.

Materials and methods

3. MATERIALS AND METHODS

This chapter explains the materials used and methods adopted in the research programme entitled ‘Microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)’.

3.1 LOCATION

The study was conducted from January 2021 to October 2021 at Pesticide Residue Testing Laboratory, Department of Agricultural Entomology, and at Agri Business Incubator, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur.

3.2 TEST INSECT

The nucleus culture of a susceptible strain of test insect, *Tribolium castaneum* was procured from the Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, which was maintained without exposure to any insecticide for more than 35 years. Adults from nucleus culture were mass multiplied for the various experiments.

3.2.1 Rearing of test insect

The culture of *T. castaneum* was maintained in the laboratory on whole wheat flour and 5 per cent brewer’s yeast at 30°C and 70 per cent relative humidity. Wheat grains procured from the local market were thoroughly washed in tap water to remove impurities and pesticides if any. The washed grains were sundried and then ground to fine flour. The flour was sterilized in a hot air oven at 55°C for one hour and stored in airtight plastic containers. The moisture content of sterilized wheat flour was estimated to be 10.63 per cent. The adults of red flour beetle from the nucleus culture were released into PET jars of 1 L capacity containing 100 g flour and five g brewer’s yeast. Then the jars were covered with muslin cloth and secured with rubber bands. After labelling the date of inoculation, they were kept for incubation in rearing racks (Plate 1). Adults were sieved out after five days and transferred to fresh rearing jars to obtain uniformly aged test insects for conducting various experiments.

3.3 SOURCE OF IRRADIATION

A household microwave oven (230 V, 50 Hz, Morphy Richards 20 MS) available at the KAU Agribusiness Incubator, Department of Agricultural Engineering, College of Agriculture, Vellanikkara, Thrissur was used in the study (Plate 2). The frequency of microwaves passing through the oven is 2450 MHz. The 20 L capacity oven works at five different power levels *i.e.*, low (136 W), medium-low (264 W), medium (440 W), medium-high (616 W) and high (800 W). Knobs for adjusting the power and exposure time are present. Samples (Plate 3) were placed on the turntable for treatment which rotates clockwise or anticlockwise.

3.4 SUSCEPTIBILITY OF RED FLOUR BEETLE EGGS TO MICROWAVE RADIATION

Adult beetles were introduced into a rearing jar containing wheat flour for egg laying. The flour was previously sieved through B.S.S 60 sieve (0.25 mm) to ensure only eggs (0.3 mm size) were retained on the sieve (Plate 4). Sifting the culture the next day through a B.S.S. 18 (0.85 mm) sieve separated the released adults (Plate 5). The flour containing eggs were held for another day to obtain two-day old eggs (Plate 6) required for the treatment.

The two-day old eggs were transferred to 50 ml glass beakers containing wheat flour filled at five bed thicknesses (1, 2, 3, 4, 5 cm). Five eggs were released to each beaker. The prepared samples were exposed to microwave radiation at five levels (136, 264, 440, 616, 800 W) by varying the exposure time at six different levels (10, 20, 30, 40, 50, 60 s). Five replications were kept for each treatment combination. In addition, five replications were kept unirradiated as control. The larvae that emerged from the irradiated and unirradiated eggs were observed two weeks after treatment by sieving the samples through B.S.S 60 sieve. This data was used to calculate the mortality of eggs.



Plate 1. Test insects culture



Plate 2. Microwave oven



Plate 3. Samples prepared for irradiation



Plate 4. B.S.S 60 sieve



Plate 5. B.S.S. 18 sieve

Per cent egg mortality

The per cent egg mortality was calculated using the formula,

$$\begin{aligned} \text{Egg mortality (\%)} \\ &= \frac{(\text{Number of eggs released} - \text{Number of eggs hatched})}{\text{Total number of eggs released}} \times 100 \end{aligned}$$

3.5 SUSCEPTIBILITY OF RED FLOUR BEETLE GRUBS TO MICROWAVE RADIATION

To study the susceptibility of grubs of red flour beetle to microwave radiation, 20±2 day old grubs were selected. Adult beetles were released to fresh rearing bottles containing wheat flour and allowed to oviposit. On the next day, the flour was sieved to separate the released adults. The flour containing one-day old eggs were observed for hatching. The eggs hatched on the subsequent five days were separately kept for another 17 days to get uniformly aged (20±2 day old) grubs (Plate 7).

The 20±2 day old grubs were transferred to 50 ml glass beakers containing wheat flour filled at five bed thicknesses (1, 2, 3, 4, 5 cm). Five grubs were released to each beaker. The prepared samples were exposed to microwave radiation at five different levels (136, 264, 440, 616, 800 W) by varying the exposure time at six different levels (10, 20, 30, 40, 50, 60 s) along with an unirradiated control. Five replications were kept for each treatment combination. The treated samples were observed after five days for pupation, and per cent mortality of grubs was calculated.

Per cent mortality of grubs

Per cent mortality of grubs was calculated using the equation,

$$\begin{aligned} \text{Grub mortality (\%)} \\ &= \frac{(\text{Number of grubs released} - \text{Number of pupae formed})}{\text{Number of grubs released}} \times 100 \end{aligned}$$

3.6 SUSCEPTIBILITY OF RED FLOUR BEETLE PUPAE TO MICROWAVE RADIATION

The pupation of red flour beetle commenced 20-24 days after larval emergence. The pupae (Plate 8) sieved out from the culture maintained as described in section 3.2.1 were transferred to 50 ml glass beakers containing wheat flour filled at five bed thicknesses (1, 2, 3, 4, 5 cm). Five pupae were released to each beaker. The prepared samples were exposed to microwave radiation at five levels (136, 264, 440, 616, 800 W) by varying the exposure time at six different levels (10, 20, 30, 40, 50, 60 s). Five replications were kept for each treatment combination along with control. The treated and control samples were observed after five days for adult emergence, and per cent adult emergence was calculated.

Per cent pupal mortality

The total number of adults emerged from microwave irradiated samples of pupae was observed to calculate the per cent pupal mortality.

Pupal mortality (%)

$$= \frac{(\text{Number of pupae irradiated} - \text{Number of adults emerged})}{\text{Number of pupae irradiated}} \times 100$$

3.7 SUSCEPTIBILITY OF RED FLOUR BEETLE ADULTS TO MICROWAVE RADIATION

To study the susceptibility of microwave radiation on adults of red flour beetles, 17±2 day old adults (Plate 9) were used for the experiment. Adult beetles were released to rearing jars for egg laying and sub-cultured after five days. The rearing jars were frequently examined for adult emergence. The adults that emerged in the five days following their first appearance were separated and held for another 14 days to produce consistently aged (17±2 day old) adults.

The adult beetles collected were transferred to 50 ml glass beakers containing wheat flour at five bed thicknesses (1, 2, 3, 4, 5 cm). Five adults were released to each

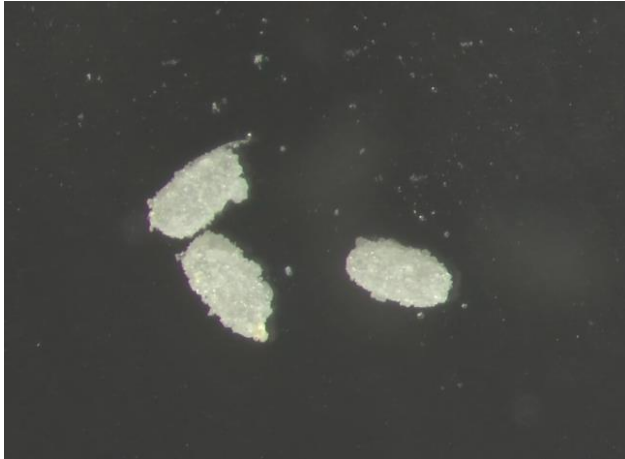


Plate 6. Two day old eggs



Plate 7. 20±2 day old grub



Plate 8. Pupa



Plate 9. 17±2 day old adult

beaker. The prepared samples were exposed to microwave radiation at five different levels (136, 264, 440, 616, 800 W) by varying the exposure time at six different levels (10, 20, 30, 40, 50, 60 s). Five replications were kept for each treatment combination. The mortality of adults in each treatment combination after irradiation was observed two days after treatment by sieving the samples through B.S.S 18 sieve. This data was used to calculate the per cent mortality of adults.

Per cent adult mortality

Per cent adult mortality was calculated using the formula,

$$\text{Adult mortality (\%)} = \frac{\text{Total number of adults died}}{\text{Total number of adults released for treatment}} \times 100$$

3.8 EFFECT OF MICROWAVE IRRADIATION ON NUTRITIONAL FACTORS OF WHEAT FLOUR

The effect of microwave radiation on the physical and nutritional factors of wheat flour was analysed. The best treatment combinations which ensured complete disinfestation of all the stages of red flour beetle after microwave treatment were selected and analysed for gluten content (AOAC, 1980), vitamin B content (Setiawan, 2010), moisture content (Obi *et al.*, 2016) and colour (Mohapatra *et al.*, 2019).

3.8.1 Gluten content of wheat flour

Gluten of irradiated and non-irradiated wheat flour samples were estimated by hydrating it with water and then washing it to remove starch, sugars, water-soluble proteins and other minor components. The wet cohesive viscoelastic product, free of starch, will be wet gluten and is dried to get dry gluten.

Twenty-five g of irradiated wheat flour was weighed and kept in a plastic bowl. Fifteen ml of water was added to the sample and kneaded the flour to make consistent dough. Then the dough was immersed in water and kept for one hour. After one hour, the dough was washed to remove starch in a slow stream of water by keeping a fine sieve beneath. The washing was continued till the washed out liquid became clear,

indicating the complete removal of starch. The cohesive mass thus obtained was wet gluten, and was dried in a hot air oven at 100 °C for 24 h to obtain dry gluten.

$$\text{Dry gluten (\%)} = \frac{\text{Weight of dry gluten}}{\text{Weight of wheat flour sample taken}} \times 100$$

3.8.2 Estimation of moisture content (Hot air oven method)

Ten g of flour sample was weighed and transferred to a previously weighed sample box (W_1). The samples were then kept in a hot air oven at 105 °C for 24 hours or until the weight of the sample became constant (Plate. 10). After 24 hours, the sample was weighed along with the sample box (W_2).

$$\text{Final weight of sample} = W_2 - W_1$$

$$\begin{aligned} \text{Moisture content of sample} \\ = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100 \end{aligned}$$

3.8.3 Estimation of colour

Hunter Lab miniscan (Plate 11) was used to estimate L (lightness), a (redness), b (yellowness) colour values of wheat flour. White and black tiles were used to calibrate the device. The colour values of irradiated (L^* , b^* , a^*) as well as control (L , a , b) samples were measured. Two replications were kept for each treatment, and colour change was calculated using the following formula.

$$\text{Colour change } (\Delta E) = \sqrt{[(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2]}$$

3.8.4 Estimation of vitamin B

Ten g of wheat flour was weighed into 100 ml volumetric flask, and 100 ml water was added. A minimum amount of 0.25 M NaOH was added to enhance the solubility issue of some vitamins. After 15 min of ultrasonic extraction, samples were centrifuged at 4,500 rpm. The supernatant obtained was filtered through 0.4 μm filter, and the samples were injected in the HPLC system according to the conditions in Table 1.

3.9 ANALYSIS OF DATA

The data obtained through the experiments were analysed in factorial completely randomized design using a R based statistical package, GRAPES after arc sine transformation (Gopinath *et al.*, 2020).

Table 1. HPLC parameters for the analysis of vitamin B

Sl.no.	Parameter	Conditions		
1	Column	Shimpack 4.6 X 150mm, 3 μ m		
2	Column oven temperature	35°C		
3	Data acquisition	25 min		
4	Injection volume	20 μ L		
5	Flow rate	0.4 ml/min		
6	Sample thermostat	5 °C		
7	Mobile phase A	25 mM HK ₂ PO ₄ (pH-7.0)		
8	Mobile phase B	Acetonitrile		
9	Gradient	Time	Command	Value
		0.01	B. Conc.	1
		5.00	B. Conc.	1
		15.00	B. Conc.	30
		20.00	B. Conc.	30
		20.10	B. Conc.	1
		25.00	B. Conc.	1
		25.00	Stop	
10	Post run time	5 min		



Plate 10. Hot air oven



Plate 11. Hunter MiniScan

Results

4. RESULTS

Microwave radiation is a physical curative method for the management of storage pests. The research studied the effect of the power of microwave radiation, flour bed thickness and duration of irradiation on various life stages of *T. castaneum*. The result thus obtained is presented in this chapter. After statistical analysis, the main effects and the interaction effects are presented separately for each life stage of *T. castaneum*.

4.1 EFFECT OF MICROWAVE RADIATION ON EGGS OF *Tribolium castaneum*

4.1.1 Effect of power of microwave radiation on two-day-old eggs of *Tribolium castaneum*

Microwave radiation resulted in dose-dependent mortality of two-day-old eggs of *T. castaneum* (Table 2). The highest power of 800 W caused significantly higher egg mortality of 94 per cent over all other doses. At 616 W, mortality was 88.67 per cent, significantly higher than all the other lower doses. All the eggs hatched in the unirradiated control. Even at the lowest dose of 136 W, there was significantly higher mortality of 18.27 per cent over control. The egg mortality at 264 W and 440 W were 54.13 per cent and 77.87 per cent, respectively, which differed significantly among each other and from other treatments.

4.1.2 Effect of flour bed thickness on two-day-old eggs of *Tribolium castaneum*

The flour bed thickness had a negative influence on the mortality of two-day-old eggs of *T. castaneum*, *i.e.*, as the flour bed thickness increased, the egg mortality decreased (Table 3). The egg mortality at the lowest thickness of one cm was 67.11 per cent, which was significantly higher when compared to all other flour bed thicknesses. There was comparable egg mortality of 57.89 and 55.44 per cent at flour bed thickness of two cm and three cm, which were significantly superior to mortality at other higher thicknesses. At flour bed thickness of four cm and five cm, the egg mortality was 51.11 and 45.89 per cent, respectively, which differed significantly from each other and other treatments.

Table 2. Effect of power of microwave radiation on two-day old eggs of *Tribolium castaneum*

Power (W)	Egg mortality (%)
Control	0.00 ^f (0.23)
136	18.27 ^c (0.44)
264	54.13 ^d (0.83)
440	77.87 ^c (1.10)
616	88.67 ^b (1.22)
800	94.00 ^a (1.28)

*Figures in the parentheses are arc sine transformed values
 Values with the same letters are not significantly different at 5.00% level by LSD

Table 3. Effect of flour bed thickness on two-day old eggs of *Tribolium castaneum*

Flour bed thickness (cm)	Egg mortality (%)
1	67.11 ^a (0.98)
2	57.89 ^b (0.88)
3	55.44 ^b (0.85)
4	51.11 ^c (0.80)
5	45.89 ^d (0.74)

*Figures in the parentheses are arc sine transformed values
 Values with the same letters are not significantly different at 5.00% level by LSD

4.1.3 Effect of exposure time on two-day-old eggs of *Tribolium castaneum*

The mortality of eggs of *T. castaneum* was directly proportional to the exposure time of microwave radiation (Table 4). The mortality rate (70.53 per cent) was significantly higher at 60 s compared to other exposure times. The egg mortality at 50 (67.33 per cent), 40 (61.87 per cent), 30 (56.53 per cent), and 20 s (48.27 per cent) significantly differed from each other and were superior to the mortality at the lowest exposure time of 10 s (28.40 per cent).

4.1.4 Interaction effect of power and thickness on two-day-old eggs of *Tribolium castaneum*

If we analyse the interaction effect of power and thickness of flour bed on egg mortality of *T. castaneum*, it is evident that significantly higher mortality occurred at the highest dose and lower flour bed thickness (Table 5). At the lowest flour bed thickness of one cm and the two higher doses of 616 W and 800 W, egg mortality was a significantly higher (99.33 per cent). At the highest dose of 800 W, even though we increased the flour bed thickness to two cm (97.33 per cent) and three cm (95.33 per cent), there was no significant difference in mortality from the lowest thickness of one cm. But, an increase in flour bed thickness to four and five cm decreased the egg mortality significantly to 91.33 per cent and 86.67 per cent. It significantly differed from mortality at 616 W and 800 W with flour bed thickness of one cm. However, the mortality at four cm did not differ significantly from mortality at two cm and three cm at 800 W.

Egg mortality at 616 W at the flour bed thickness of two, three, four and five cm was 88.00, 86.67, 85.33 and 84.00 per cent, respectively, which did not differ significantly from egg mortality at 440 W at one cm flour bed thickness. Egg mortality decreased from 80.00 per cent at two cm flour bed thickness, at 440 W to 65.33 per cent at five cm flour bed thickness, and they differed significantly. But, the egg mortality at three cm and four cm flour bed thickness did not vary significantly from flour bed thickness of two cm at 440 W.

Table 4. Effect of exposure time on two-day old eggs of *Tribolium castaneum*

Exposure time (s)	Egg mortality (%)
10	28.40 ^f (0.55)
20	48.27 ^e (0.77)
30	56.53 ^d (0.86)
40	61.87 ^c (0.92)
50	67.33 ^b (0.98)
60	70.53 ^a (1.02)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 5. Interaction effect of power and thickness on two-day-old eggs of *Tribolium castaneum*

Power (W)	Thickness (cm)				
	1	2	3	4	5
Control	0.00 ^o (0.23)	0.00 ^o (0.23)	0.00 ^o (0.23)	0.00 ^o (0.23)	0.00 ^o (0.23)
136	34.00 ^{kl} (0.61)	23.33 ^m (0.49)	12.67 ⁿ (0.37)	12.00 ⁿ (0.37)	9.33 ⁿ (0.34)
264	81.33 ^{fghi} (1.14)	58.67 ^j (0.89)	59.33 ^j (0.89)	41.33 ^k (0.69)	30.00 ^{lm} (0.56)
440	88.67 ^{cde} (1.22)	80.00 ^{ghi} (1.12)	78.67 ^{hi} (1.11)	76.67 ⁱ (1.08)	65.33 ^j (0.96)
616	99.33 ^a (1.34)	88.00 ^{cdef} (1.21)	86.67 ^{defg} (1.20)	85.33 ^{defgh} (1.18)	84.00 ^{efgh} (1.17)
800	99.33 ^a (1.34)	97.33 ^{ab} (1.32)	95.33 ^{abc} (1.29)	91.33 ^{bcd} (1.25)	86.67 ^{defg} (1.20)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Egg mortality at 264 W with a flour bed thickness of two (58.67 per cent) and three cm (59.33 per cent) did not differ significantly from mortality at a higher power of 440 W at the highest flour bed thickness of five cm (65.33 per cent). The egg mortality at four cm flour bed thickness with 264 W and one cm flour bed thickness with 136 W are comparable; which means a higher dose of 264 W can inhibit the development of egg at a higher flour bed thickness of four cm while at the lowest power of 136 W, comparable mortality could be achieved only with lowest flour bed thickness of one cm.

The egg mortality at one cm (34.00 per cent) and two cm (23.33 per cent) flour bed thickness at 136 W were comparable to mortality at 264 W at the highest flour bed thickness of five cm (30.00 per cent). There was no significant difference in mortality of eggs at three, four and five cm flour bed thickness at the lowest dose of 136 W. At each flour bed thickness, egg mortality significantly increased with the increase in power of microwave radiation.

4.1.5 Interaction effect of power and time on two-day-old eggs of *Tribolium castaneum*

The analysis on the interaction effect of power and time on egg mortality of *Tribolium castaneum* indicated that significantly higher mortality occurred at higher power and exposure time (Table 6). At highest doses of 616 and 800 W and three higher exposure times of 40, 50 and 60 s, egg mortality was 100 per cent. Even at medium power of 440 W, there was complete mortality of eggs by increasing the irradiation time to 60 s. All the eggs in control hatched out, so egg mortality in control was zero.

The egg mortality at 800 W power, exposed for 20 s (96.00 per cent) and 30 s (99.20 per cent), were statistically comparable to mortality at 616 W and 30 s (97.60 per cent) exposure time. They were again statistically on par to egg mortality at 440 W, at exposure times of 40 s (95.20 per cent) and 50 s (96.80 per cent) and at 264 W treated for 60 s (92.00 per cent). Thus, treatment at high powers (800, 616 W) for lower exposure time can give comparable mortality with treatment at comparatively lower doses (440 W, 264 W) for a higher period of exposure. Egg mortality at the

lowest exposure of 10 s, at 800 W (68.00 per cent) and 616 W (48.00 per cent) differed significantly from each other and also from all other higher exposure times and corresponding power levels.

At 264 W, when the treatment time increased from 10 s to 50 s, the egg mortality gradually increased from 15.20 to 82.40 per cent, and they differed significantly among each other. At 136 W, even though egg mortality increased with exposure time, mortality at 10 s was on par with mortality at 20 s, which in turn was comparable to mortality at 30 and 40 s.

Egg mortality at a medium dose of 440 W, at 10 s (32.00 per cent) did not statistically differ from mortality at 136 W, at two different exposure times of 50 (24.80 per cent) and 60 s (31.20 per cent). Hence, there was appreciable and comparable egg mortality even at the lower microwave power (136 W), as exposure time was longer (50 and 60 s).

At 10 s and 20 s exposure, mortality of eggs steadily increased with increase in power of microwave radiation and they significantly differed among all the doses. Similarly, at 40, 50 and 60 s treatment times, egg mortality increased proportionally with power until maximum mortality of 100 per cent was achieved.

4.1.6 Interaction effect of thickness and time on two-day-old eggs of *Tribolium castaneum*

The study on the interaction effect of thickness and time on mortality of two-day-old eggs of *T. castaneum* indicated that there was significantly higher egg mortality when the exposure time increased and flour bed thickness decreased (Table 7). At the thickness -time combination of one cm and 60 s, there was considerably higher mortality of 79.33 per cent, which was statistically on par with mortality at flour bed thickness of two cm, at 50 (74.67 per cent) and 60 s (74.67 per cent) and one cm flour bed thickness with a treatment time of 50 s (74.00 per cent).

Egg mortality at one cm flour bed thickness treated for 20 (67.33 per cent), 30 (70.67 per cent) and 40 s (69.33 per cent) were on par but significantly differed from mortality at 10 s treatment time (42.00 per cent). They were also comparable with

Table 6. Interaction effect of power and time on two-day-old eggs of *Tribolium castaneum*

Power (W)	Time (s)					
	10	20	30	40	50	60
Control	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)
136	6.40 ^{lm} (0.30)	12.00 ^{kl} (0.37)	18.40 ^{jk} (0.44)	16.80 ^{jk} (0.42)	24.80 ^{ij} (0.51)	31.20 ^{hi} (0.58)
264	15.20 ^k (0.40)	32.80 ^h (0.60)	43.20 ^g (0.71)	59.20 ^f (0.89)	82.40 ^d (1.14)	92.00 ^{bc} (1.25)
440	32.00 ^{hi} (0.59)	62.40 ^{ef} (0.92)	80.80 ^d (1.13)	95.20 ^{ab} (1.29)	96.80 ^{ab} (1.31)	100.00 ^a (1.35)
616	48.00 ^g (0.77)	86.40 ^{cd} (1.19)	97.60 ^{ab} (1.32)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
800	68.80 ^e (0.995)	96.00 ^{ab} (1.30)	99.20 ^{ab} (1.34)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with same letters are not significantly different at 5.00% level by LSD

Table 7. Interaction effect of thickness and time on two-day-old eggs of *Tribolium castaneum*

Thickness (cm)	Time (s)					
	10	20	30	40	50	60
1	42.00 ^l (0.70)	67.33 ^{def} (0.98)	70.67 ^{bcd} (1.02)	69.33 ^{bcde} (1.00)	74.00 ^{abc} (1.05)	79.33 ^a (1.11)
2	31.33 ^m (0.58)	45.33 ^{kl} (0.74)	56.67 ^{ij} (0.86)	64.67 ^{defgh} (0.95)	74.67 ^{abc} (1.06)	74.67 ^{abc} (1.06)
3	30.00 ^m (0.56)	51.33 ^{jk} (0.80)	56.00 ^{ij} (0.85)	60.67 ^{ghi} (0.91)	66.67 ^{defg} (0.97)	68.00 ^{cdef} (0.99)
4	19.33 ⁿ (0.44)	45.33 ^{kl} (0.83)	56.67 ^{ij} (0.86)	59.33 ^{hi} (0.89)	59.33 ^{hi} (0.89)	66.67 ^{defg} (0.97)
5	19.33 ⁿ (0.45)	32.00 ^m (0.58)	42.67 ^l (0.70)	55.33 ^{ij} (0.85)	62.00 ^{fghi} (0.92)	64.00 ^{efgh} (0.94)

*Figures in the parentheses are arc sine transformed values

Treatments with same letters are not significantly different at 5.00% level by LSD

mortalities at higher thickness such as three and four cm, *i.e.*, 68.00 and 66.67 per cent respectively, treated at the longest exposure period of 60 s. Thus, an appreciable and comparable reduction in the hatchability of eggs occurs at lower thickness exposed for a shorter duration and at higher thicknesses with longer exposure time.

Egg mortality at two and five cm flour bed thickness significantly differed at each exposure time from 10 to 40 s. But at three and four cm flour bed thickness, even though the egg mortality at 40 s was significantly different from 10 s, it was comparable with mortality at 30 s. At flour bed thicknesses of one, two, three and five cm, there was a comparable inhibition in the development of eggs even if we increased the treatment time from 50 to 60 s. Egg mortality was 19.33 per cent in higher flour bed thickness of four and five cm at the lowest exposure time of 10 s, which was the lowest recorded mortality in this interaction effect

The mortality of eggs between the three flour bed thicknesses of two, three and four cm were comparable when irradiated for 20 and 30 s, but significantly differed from mortalities at one cm and five cm. But, at 10 s exposure time, egg mortalities at two and three cm were on par but differed significantly to mortality at one, four and five cm.

4.1.7 Interaction effect of power, thickness and time on two-day-old eggs of *Tribolium castaneum*

If we compare the interaction of power, bed thickness and time, the mortality of eggs increases as we increase the power of irradiation, reduce the thickness of the flour bed and increase the exposure period (Table 8). At a flour thickness of one cm, 100 per cent mortality could be observed with 20 s and 30 s exposure at 616 and 800 W. However, as the exposure time increased to 40 and 50 s, there was 100 per cent mortality at a lower dose of 440 W, while at 60 s exposure, 100 per cent mortality occurred at the second-lowest dose of 264 W. At a power of 264 W with an exposure time of 20 (96.00 per cent), 30 (96.00 per cent), 40 (88.00 per cent), and 50 s (96.00 per cent), mortality did not significantly differ from the cent per cent mortality observed at higher doses and longer exposure time. The exposure time of 10 s could not cause any significant mortality even at a power of 264 W with egg mortality in control (0.00 per cent).

Complete inhibition in egg hatchability at two cm flour bed thickness could be observed at the same power-time combinations at one cm flour thickness. At 440 and 264 W power and at 30 (92.00 per cent) and 50 s (96.00 per cent) exposure time, respectively, egg mortality was on par with treatment combinations with cent per cent mortality. Egg mortality was cent per cent at all the doses except the lowest dose of 136 W when exposed to irradiation for 60 s. Whereas complete inhibition of egg development occurred at 440, 616 and 800 W when exposed for 40 and 50 s. But, reducing the exposure to 20 and 30 s resulted in complete mortality of eggs only at the higher two doses of 616, and 800 W. An exposure of 10 s to microwave radiation did not cause complete mortality even at the highest dose. Mortality at 136 W after 10 (8.00 per cent), 20 (4.00 per cent), and 30 s (12.00 per cent) exposure was statistically similar to mortality at control (0.00 per cent).

At flour thickness of three cm and 136 W irradiation power, egg mortality at all treatment times was significantly low compared to mortality at higher doses and similar to control. Whereas there was 100 per cent mortality at higher doses of 264, 440, 616 and 800 W at 60s; 50 and 60 s; 30 to 60 s and 20 to 60 s, respectively. They were comparable with egg mortality at 264 (96.00 per cent) and 440 W (92.00 per cent), irradiated for 50 and 40 s, respectively.

At four cm flour bed thickness, irradiated at the highest dose of 800 W, all the eggs treated at 20 s onwards died. At a lower dose of 616 W, egg mortality was complete from 30 s onwards only. At 400 W, only the longest exposure time of 60 s, resulted in the total mortality of eggs. At 264 W, at 60 s exposure time, egg mortality was 88.00 per cent, which was comparable with cent per cent mortality attained at higher doses and with 92.00 per cent mortality observed at 440 W with 40 and 50 s exposure. Mortality with 136 W at all the exposure periods and with 264 W at 10 s exposure was comparable with 0.00 per cent mortality at control.

At the highest flour bed thickness of five cm, total mortality of eggs occurred only at higher doses of 440 W treated for 60 s, and at 616 and 800 W, irradiated for 40 to 60 s. Exposing eggs for 30 s at 616 W (88.00 per cent) and 800 W (96.00 per cent), 40 (92.00 per cent), and 50 s (92.00 per cent) at 440 W did not differ significantly

from cent per cent mortality achieved with various treatment combinations. At 264 W, even at 60 s time, egg mortality was 72.00 per cent only. At 136 W, mortality at all exposure times was significantly lower but comparable to mortality in control (0.00 per cent). Similarly, mortality at 264 W with 10, 20 and 30 s exposure did not differ from control.

In brief, exposure of eggs of *T. castaneum* at 136 W was not sufficient to cause complete mortality of eggs with none of the treatment combinations. Irradiation at 616 and 800 W resulted in a cent per cent mortality of eggs with an exposure of 20 and 30 s at one and two cm flour thickness. At flour thickness of three and four cm, with 20 s exposure, only 800 W power caused cent per cent mortality of eggs. But, increasing the exposure to 30 s caused 100 per cent mortality with 616 W power also. Exposure to 440 W of microwave radiation was sufficient to cause total mortality of eggs at one and two cm flour bed thickness with an exposure of 40 and 50 s. In contrast, raising the flour thickness to three cm resulted in the complete mortality of eggs by increasing the exposure to 50 s. Power of 264 W was sufficient to cause complete mortality of eggs with 60 s exposure up to three cm flour bed thickness. Irradiating at 616 W was sufficient to cause 100 per cent mortality of eggs with exposure for 50 s at flour bed thickness of four and five cm. Raising the exposure to 60 s and reducing the power of irradiation to 440 W, resulted in cent per cent at a bed thickness of four and five cm.

4.2 EFFECT OF MICROWAVE RADIATION ON GRUBS OF *Tribolium castaneum*

4.2.1 Effect of power of microwave radiation on 20±2 day old grubs of *Tribolium castaneum*

Microwave radiation caused the death of 20±2 day old grubs of *T. castaneum* in a dose-dependent manner (Table 9). Mortality at each irradiation power differed significantly from each other and with control. The grub mortality at 800 W (90.80 per cent) was superior to all the other doses and control. There was no mortality of released grubs recorded in control. At 616 W, the mortality of grubs was 84.53 per cent which was significantly higher than all other lower doses but inferior to the highest dose. The microwave irradiation at 440, 264 and 136 W caused grub mortality at the rate of 78.67, 50.53 and 18.13 per cent, respectively (Plate 12).

4.2.2 Effect of flour bed thickness on 20±2 day old grubs of *Tribolium castaneum*

Microwave treatment of 20±2 day old grubs of *Tribolium castaneum* at thin layers of wheat flour gave higher mortality than thicker layers (Table 10). At flour bed thickness of one cm, there was significantly higher grub mortality of 69.44 per cent compared to the remaining thicknesses. At two cm flour bed thickness, grub mortality was 58.33 per cent, which was the next best treatment after one cm. Grub mortality was comparable at flour bed thickness of three (50.33 per cent) and four cm (49.22 per cent) but significantly higher than mortality at five cm flour bed thickness (41.56 per cent).

4.2.3 Effect of microwave exposure time on 20±2-day old grubs of *Tribolium castaneum*

The results on the impact of irradiation exposure time on mortality of 20±2-day old grubs of *T. castaneum* indicated that as we increased the exposure time, grub mortality increased significantly from 10 to 60 s (Table 11). The mortality at 50 s (65.47 per cent) was on par with mortality at 40 s (62.93 per cent) and superior to mortality at lower exposure time but inferior to mortality at 60 s. When we exposed the grubs to microwave radiation for 20 and 30 s, grub mortality was 49.33 and 56.80 per cent, and they differed statistically from each other and with all other treatments.

4.2.4 Interaction effect of power and thickness on 20±2-day old grubs of *Tribolium castaneum*

Microwave irradiation at higher power and lower bed thickness adversely affected the pupation of 20±2-day old grubs of *T. castaneum* (Table 12). The grub mortality was highest and comparable when irradiated at the higher dose of 800 W by keeping the thickness of flour bed at thin layers of one cm (100.00 per cent) and two cm (98.00 per cent). There was comparable mortality of grubs irradiated at 800 W, at flour bed thickness of three cm (86.00 per cent) and four cm (88.67 per cent) with microwave treatment at 616 W and flour bed thickness of one (87.33 per cent), two (88.00 per cent), three (84.00 per cent) and four cm (85.33 per cent). They did not differ significantly with grub mortality at 440 W and flour bed thickness of one (85.33 per cent) and two cm (85.33 per cent) and at 264 W, at one cm (86.67 per cent) flour bed thickness, which indicates that to achieve higher grub mortality at lower doses, flour bed thickness should be reduced.

Table 8. Interaction effect of power, thickness and time on two-day-old eggs of *Tribolium castaneum*

Thickness (cm)	Time (s)	Power (W)					
		Control	136	264	440	616	800
1	10	0.00 ^z (0.23)	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	48.00 ^{ijklmn} (0.77)	96.00 ^{ab} (1.30)	96.00 ^{ab} (1.30)
	20	0.00 ^z (0.23)	16.00 ^{uvwxy} (0.41)	96.00 ^{ab} (1.30)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^z (0.23)	36.00 ^{lmnopq} (0.64)	96.00 ^{ab} (1.30)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	28.00 ^{opqrstu} (0.55)	88.00 ^{abcd} (1.21)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	48.00 ^{ijklmn} (0.77)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	76.00 ^{defg} (1.07)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
2	10	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	28.00 ^{opqrstu} (0.55)	40.00 ^{klmnopq} (0.68)	28.00 ^{opqrstu} (0.55)	84.00 ^{bcde} (1.17)
	20	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	20.00 ^{stuvw} (0.45)	48.00 ^{ijklmn} (0.77)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	36.00 ^{mnpqrs} (0.63)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	16.00 ^{uvwxy} (0.42)	72.00 ^{defg} (1.04)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	52.00 ^{ijklm} (0.80)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	48.00 ^{ijklmn} (0.76)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
3	10	0.00 ^z (0.23)	16.00 ^{uvwxy} (0.41)	28.00 ^{opqrstu} (0.55)	24.00 ^{qrstuvw} (0.50)	40.00 ^{klmnopq} (0.68)	72.00 ^{efgh} (1.02)
	20	0.00 ^z (0.23)	20.00 ^{rstuvw} (0.46)	32.00 ^{nopqrst} (0.59)	76.00 ^{defg} (1.07)	80.00 ^{cdef} (1.11)	100 ^a (1.35)
	30	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	44.00 ^{klmnop} (0.72)	80.00 ^{cdef} (1.11)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	16.00 ^{uvwxyz} (0.41)	56.00 ^{hijk} (0.85)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

4	10	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	0.00 ^z (0.23)	28.00 ^{pqrstuv} (0.55)	36.00 ^{lmnopqr} (0.63)	48.00 ^{jklmn} (0.77)
	20	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	16.00 ^{uvwxy} (0.41)	68.00 ^{fghi} (0.98)	76.00 ^{defg} (1.07)	100.00 ^a (1.35)
	30	0.00 ^z (0.23)	24.00 ^{qrstuvw} (0.51)	36.00 ^{lmnopqr} (0.63)	80.00 ^{cdef} (1.11)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	52.00 ^{ijkl} (0.81)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	56.00 ^{hijk} (0.85)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	88.00 ^{abcd} (1.21)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
5	10	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	8.00 ^{xyz} (0.32)	20.00 ^{tuvwxy} (0.45)	40.00 ^{klmnopq} (0.68)	44.00 ^{jklmno} (0.73)
	20	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	0.00 ^z (0.23)	28.00 ^{opqrstu} (0.55)	76.00 ^{defg} (1.07)	80.00 ^{cdef} (1.11)
	30	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	4.00 ^{yz} (0.27)	60.00 ^{ghij} (0.90)	88.00 ^{abcd} (1.21)	96.00 ^{ab} (1.30)
	40	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	28.00 ^{opqrstu} (0.55)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	68.00 ^{fghi} (0.98)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	12.00 ^{vwxyz} (0.37)	72.00 ^{efgh} (1.02)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with same letters are not significantly different at 5.00% level by LSD

Table 9. Effect of power of microwave radiation on 20±2 day old grubs of *Tribolium castaneum*

Power (W)	Mortality of grub (%)
Control	0.00 ^f (0.23)
136	18.13 ^e (0.43)
264	50.53 ^d (0.79)
440	78.67 ^c (1.11)
616	84.53 ^b (1.17)
800	90.80 ^a (1.24)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 10. Effect of flour bed thickness on 20±2 day old grubs of *Tribolium castaneum*

Flour bed thickness (cm)	Mortality of grub (%)
1	69.44 ^a (1.00)
2	58.33 ^b (0.88)
3	50.33 ^c (0.79)
4	49.22 ^c (0.78)
5	41.56 ^d (0.69)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 11. Effect of microwave exposure time on 20±2-day old grubs of *Tribolium castaneum*

Exposure time (s)	Mortality of grub (%)
10	18.33 ^c (0.44)
20	49.33 ^d (0.78)
30	56.80 ^c (0.86)
40	62.93 ^b (0.93)
50	65.47 ^b (0.96)
60	69.60 ^a (1.00)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Grub mortality significantly decreased with the increase in flour bed thickness from one cm to five cm at all irradiation power. There was significantly higher mortality of 57.33 per cent at 136 W and one cm flour bed thickness, as compared to higher thickness. Irradiating the grubs at 264 W at five cm flour bed thickness resulted in the death of 26.00 per cent grubs, which was on par with mortality at four cm flour bed thickness. Comparable grub mortality can be observed between thicknesses from one cm to four cm at 440 and 616 W power levels. At 440 W and five cm flour bed thickness, the grub mortality was 62.00 per cent, and it was on par with mortality at the lowest dose of 136 W, at one cm thickness and 264 W, at two cm flour bed thickness. There was significantly lower grub mortality of 2.67 and 2.00 per cent at the lowest dose of 136 W, correspondingly at flour bed thickness of three cm and five cm, and this was on par with mortality in control, which was zero per cent.

Grub mortality did not differ significantly between 616 W and 440 W at each flour bed thickness, except at five cm. For five cm flour bed thickness, there was 78.00 and 81.33 per cent grub mortality at 616 and 800 W, respectively, and they did not differ statistically.

4.2.5 Interaction effect of power and time on 20±2-day old grubs of *Tribolium castaneum*

If we evaluate the interaction effect of power and exposure time on the mortality of 20±2-day old grubs of *Tribolium castaneum*, it is conspicuous that significantly higher mortality occurred at higher power and longer exposure time (Table 13). There was complete mortality of grubs at higher microwave power of 616 and 800 W, irradiated for 40, 50 and 60 s, which was comparable to grub mortality with 800 W, at 20 (94.40 per cent) and 30 s (98.40 per cent), with 616 W exposed for 30 s (96.00 per cent) and with 440 W treated for 40 (93.60 per cent), 50 (94.40 per cent) and 60 s (99.20 per cent). Grub mortality at 616 W and 20 s (89.60 per cent) did not differ statistically from mortality at 440 W and 30 s (88.00 per cent), and they were on par with mortality at a lower dose of 264 W exposed for 60 s (84.00 per cent). There was a steady increase in grub mortality at 264 W, from 10.40 to 84.00 per cent with an increase in time of exposure from 10 to 60 s, and mortalities differed

significantly between each exposure time. At 136 W, the grub mortality at 60 s (34.40 per cent) was on par with mortality at 50 s (27.20 per cent), which was on par with mortality at 40 s (22.40 per cent), and they were all superior over mortalities at lower treatment times at the same irradiation power. There was comparable mortality of grubs at 136 W exposed for 40 and 50 s with mortalities at 440 and 616 W treated for the lowest time of 10 s. Grub mortality at 136 W, 10 and 20 s was 7.20 and 4.80 per cent, respectively, and was comparable to observation in control, where all the released grubs pupated.

At 10 s exposure time, even though we increased microwave radiation power, the grub mortality reached only up to 52.00 per cent. In contrast, at other higher exposure times (20 s to 60 s), mortality was more than 90.00 per cent at 800 W.

4.2.6 Interaction effect of power, thickness and time on 20±2-day old grubs of *Tribolium castaneum*

Keeping flour thickness at one cm and irradiating the grubs of *T. castaneum* at 800 W, there was complete mortality even for an exposure of 10 s (Table 14). To achieve 100 per cent grub mortality, there should be reduction in microwave power to 616 or 440 W with exposure time raised to 20 s. There was 100 per cent mortality of grubs even at a lower dose of 264 W, but with a higher exposure of 50 and 60 s. Grub mortality at 136 W with 50 (88.00 per cent) and 60 s (92.00 per cent) exposure and at 264 W with 20 (92.00 per cent), 30 (96.00 per cent) and 40 s (96.00 per cent) were comparable with the maximum mortality (100.00 per cent) attained.

If we raise the flour thickness to two cm, microwave heating at 800 and 616 W from 20 s onwards resulted in the complete death of grubs. At 440 W, there was a minimum requirement of 30 s exposure for cent per cent mortality of grubs. Microwave heating at 264 and 136 W was not enough to achieve complete grub mortality. At 264 W when irradiated for 60 s, there was comparable grub mortality of 92.00 per cent with complete mortality. Similarly, at 440 W with 20 s exposure, also caused mortality (92.00 per cent) comparable to cent per cent mortality. Grub mortality at 136 W with 10, 20 and 30 s and at 264 W with 10 s did not differ statistically from 0.00 per cent mortality in control.

Table 12. Interaction effect of power and thickness on 20±2-day old grubs of *Tribolium castaneum*

Power (W)	Thickness (cm)				
	1	2	3	4	5
Control	0.00 ^k (0.23)	0.00 ^k (0.23)	0.00 ^k (0.23)	0.00 ^k (0.23)	0.00 ^k (0.23)
136	57.33 ^f (0.86)	18.00 ⁱ (0.43)	2.67 ^k (0.26)	10.67 ^j (0.35)	2.00 ^k (0.25)
264	86.67 ^{bc} (1.19)	60.67 ^f (0.90)	48.00 ^g (0.76)	31.33 ^h (0.58)	26.00 ^h (0.52)
440	85.33 ^{bcd} (1.18)	85.33 ^{bcd} (1.18)	81.33 ^{cde} (1.14)	79.33 ^{de} (1.11)	62.00 ^f (0.92)
616	87.33 ^{bc} (1.20)	88.00 ^b (1.21)	84.00 ^{bcd} (1.16)	85.33 ^{bcd} (1.18)	78.00 ^e (1.10)
800	100.00 ^a (1.35)	98.00 ^a (1.32)	86.00 ^{bc} (1.19)	88.67 ^b (1.29)	81.33 ^{cde} (1.14)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 13. Interaction effect of power and time on 20±2-day old grubs of *Tribolium castaneum*

Power (W)	Time (s)					
	10	20	30	40	50	60
Control	0.00 ^m (0.23)	0.00 ^m (0.23)	0.00 ^m (0.23)	0.00 ^m (0.23)	0.00 ^m (0.23)	0.00 ^m (0.23)
136	7.20 ^{klm} (0.31)	4.80 ^{lm} (0.28)	12.80 ^k (0.37)	22.40 ^j (0.48)	27.20 ^{ij} (0.531)	34.40 ⁱ (0.61)
264	10.40 ^{kl} (0.34)	30.40 ⁱ (0.57)	45.60 ^h (0.74)	61.60 ^g (0.91)	71.20 ^f (1.02)	84.00 ^{de} (1.16)
440	20.00 ^j (0.45)	76.80 ^{ef} (1.08)	88.00 ^{cd} (1.21)	93.60 ^{abc} (1.27)	94.40 ^{abc} (1.28)	99.20 ^a (1.34)
616	21.60 ^j (0.47)	89.60 ^{bcd} (1.22)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
800	52.00 ^h (0.81)	94.40 ^{abc} (1.28)	98.40 ^a (1.33)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

At the highest power of 800 W, exposing grubs for 30 s was sufficient to cause cent per cent mortality at a flour thickness of three cm. As the exposure time increased to 40 s, there was cent per cent mortality at a lower dose of 440 W and 616 W. Grub mortality at 800 W with 20 s exposure and 440 W and 616 W at 30 s exposure was similar (96.00 per cent) and comparable to cent per cent mortality. At 264 W, at the highest irradiation time of 60 s, 84.00 per cent of grubs died and was significantly different from the highest mortality. Grub mortality at 136 W, 264 W irradiated for 10 and 20 s, and 440 W treated for 10 s did not differ statistically with each other and from control.

At four cm flour bed thickness, complete mortality of grubs at 800 W power occurred from an exposure of 30 s onwards. When microwave power is reduced to 616 W, treatment time should be raised to 40 s to attain cent per cent grub mortality. Microwave heating at 440 W required the highest exposure time of 60 s for the complete death of grubs. Grub mortality at 440, 616 and 800 W at 40 (92.00 per cent) and 50 s (92.00 per cent), 20 (88.00 per cent) and 30s (96.00 per cent) and 20 s (92.00 per cent), respectively, were on par with cent per cent mortality. Mortality at the lowest dose of 136 W and 264 W did not differ statistically from control mortality.

Grub mortality at the highest flour thickness (five cm) reached a maximum of 100 per cent only at higher doses of 616 and 800 W when treated for 40, 50 and 60 s. At 440 W, even at 60 s exposure, the mortality (96.00 per cent) was comparable with cent per cent mortality. Similarly, mortality at 616 W (88.00 per cent) and 800 W (92.00 per cent) was also comparable to complete mortality. At 264 W and at 60 s exposure time, mortality of 68.00 per cent grubs occurred. Still, at 136 W for the same exposure time, grub mortality (4.00 per cent) was significantly low and comparable with mortality in control. Exposing grubs for 10 s did not cause any mortality significantly higher over control and likewise, irradiating at 136 W and 264 W (up to 30 s) did not cause any mortality appreciable over control.

It can be concluded from the above data that at a lower flour thickness of one cm, there was complete mortality of grubs at the highest power of 800 W even at the lowest exposure of 10 s. At the same time, 440 W was sufficient to cause 100 per cent

grub mortality with 20, 30 and 40 s exposure. Increasing the exposure for 50 s or more led to complete mortality of grubs at 264 W. Raising the flour bed thickness to two cm reduced the mortality of grubs. Even at the highest power of 800W, we could not obtain complete mortality of grubs at the lowest exposure of 10 s. Exposure of 30 s or more to microwave radiation was sufficient to cause a cent per cent mortality of grubs at 440W. But, at 20 s exposure, complete grub mortality occurred only at 616 W. The grub mortality reduced further with an increase in flour thickness to three cm. For complete mortality of grubs, the exposure time should be raised to 40 s or more at 440 W to achieve complete mortality. At 30 s exposure, only at the highest power of 800 W, 100 per cent mortality could be achieved at three and four cm flour bed thickness. At four and five cm flour bed thickness, there was total mortality from 616 W onwards at 40 and 50 s exposure. But, we could attain 100 per cent mortality at lower power of 440 W by increasing the exposure to 60 s at four and five cm flour bed thickness.

4.3 EFFECT OF MICROWAVE RADIATION ON PUPAE OF *Tribolium castaneum*

4.3.1 Effect of power of microwave radiation on pupae of *Tribolium castaneum*

Microwave power directly impacts the mortality of pupae of *T. castaneum*. The higher the irradiation dose, the higher the pupal mortality (Table 15). The irradiation dose, which gave the highest pupal mortality of 84.67 per cent, was 800 W. At 616 W, death of 82.27 per cent pupae occurred, which was on par with mortality at 800 W. Pupae, when irradiated at 440, 264, and 136 W, resulted in mortality of 66.40, 50.13 and 27.60 per cent, respectively, and they differed significantly to each other as well as with mortality at higher doses. All the released pupae emerged into adults in control (Plate 13).

4.3.2 Effect of flour bed thickness on mortality on pupae of *Tribolium castaneum*

Flour bed thickness has a significant role in the mortality of pupae of *Tribolium castaneum* i.e., there was a gradual and significant reduction in mortality with an increase in thickness of flour bed (Table 16). The highest pupal mortality occurred at the lowest flour bed thickness of one cm (66.22 per cent) and was

significantly different from mortalities at lower thicknesses. The pupal mortality at two, three and four cm were 61.78, 50.11 and 44.89 per cent, respectively and were statistically different. The pupal mortality observed at the highest flour bed thickness (five cm) was 36.22 per cent, which was statistically different and inferior to mortalities at lower thicknesses.

4.3.3 Effect of microwave exposure time on pupae of *Tribolium castaneum*

Microwave radiation caused time-dependent mortality of pupae of *T. castaneum*. There was pupal mortality of 70.80 per cent at 60 s exposure time and was the highest recorded mortality, which was on par with mortality at 50 s (68.53 per cent) (Table 17). At 40, 30 and 20 s exposure time, the mortality of pupae was 59.07, 52.40 and 41.20 per cent, respectively, and they differed significantly among each other and with the rest of the treatments. As the pupal mortality and exposure time are directly proportional, the mortality at 10 s was the lowest (19.07 per cent).

4.3.4 Interaction effect of power and thickness on pupae of *Tribolium castaneum*

The analysis of the interaction effect of power and thickness on pupal mortality of *T. castaneum*, indicated that significantly high mortality occurred at a higher dose and lower flour bed thickness (Table 18). At one cm flour bed thickness and microwave irradiation of 616 W and 800 W, the pupal mortality was 96.67 per cent and was comparable with mortality at two cm flour bed thickness for the same power levels (88.67 per cent). The latter was comparable with mortality at 800, 616 and 440 W at three and four cm, three cm, and one cm flour bed thickness, respectively.

Pupal mortality at higher doses of 616 and 800 W at higher flour bed thickness of four (76.67 per cent) and five cm (72.67 per cent) did not differ significantly with mortality at doses of 440 (78.67 per cent) and 264 W (75.33 per cent) at lower flour bed thickness two and one cm, respectively. Hence, to obtain higher mortality at lower doses, the thickness of the flour bed should be reduced. A similar trend can be observed throughout the interaction between the power of irradiation and the thickness of flour. For example, mortality at 136 W, at one and two cm flour bed thickness was on par with mortality at 264 W at three and four cm and at 440 W at five cm.

Table 14. Interaction effect of power, thickness and time on 20±2-day old grubs of *Tribolium castaneum*

Thickness (cm)	Time (s)	Power (W)					
		Control	136	264	440	616	800
1	10	0.00 ^v (0.23)	32.00 ^{ijklmnop} (0.59)	36.00 ^{ijklmn} (0.63)	12.00 ^{qrstuv} (0.37)	24.00 ^{mnopqrst} (0.50)	100.00 ^a (1.35)
	20	0.00 ^v (0.23)	16.00 ^{opqrstuv} (0.41)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^v (0.23)	40.00 ^{ijklm} (0.67)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^v (0.23)	76.00 ^{cdef} (1.07)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^v (0.23)	88.00 ^{abcd} (1.20)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^v (0.23)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
2	10	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	4.00 ^{uv} (0.27)	20.00 ^{nopqrstu} (0.45)	28.00 ^{klmnopq} (0.56)	88.00 ^{abcd} (1.20)
	20	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	44.00 ^{ijk} (0.73)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^v (0.23)	16.00 ^{pqrstuv} (0.40)	64.00 ^{fgh} (0.94)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^v (0.23)	20.00 ^{nopqrstu} (0.45)	76.00 ^{cdef} (1.07)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^v (0.23)	28.00 ^{klmnopq} (0.56)	84.00 ^{bcde} (1.16)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^v (0.23)	36.00 ^{ijklmn} (0.63)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
3	10	0.00 ^v (0.23)	0.00 ^v (0.23)	12.00 ^{rstuv} (0.36)	12.00 ^{qrstuv} (0.37)	28.00 ^{lmnopqr} (0.56)	20.00 ^{nopqrst} (0.46)
	20	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	0.00 ^v (0.23)	80.00 ^{cdef} (1.11)	80.00 ^{bcdef} (1.11)	96.00 ^{ab} (1.30)
	30	0.00 ^v (0.23)	0.00 ^v (0.23)	36.00 ^{ijklmn} (0.63)	96.00 ^{ab} (1.30)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	40	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	84.00 ^{bcde} (1.16)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^v (0.23)	0.00 ^v (0.23)	72.00 ^{def} (1.03)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^v (0.23)	8.00 ^{stuv} (0.32)	84.00 ^{bcde} (1.16)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

4	10	0.00 ^v (0.23)	0.00 ^v (0.23)	0.00 ^v (0.23)	44.00 ^{ijkl} (0.72)	28.00 ^{klmnopq} (0.56)	40.00 ^{ijklm} (0.68)
	20	0.00 ^v (0.23)	0.00 ^v (0.23)	16.00 ^{opqrstuv} (0.41)	68.00 ^{efg} (0.99)	88.00 ^{abcd} (1.21)	92.00 ^{abc} (1.25)
	30	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	24.00 ^{mnpqrs} (0.50)	80.00 ^{bcdef} (1.12)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	40	0.00 ^v (0.23)	12.00 ^{qrstuv} (0.37)	20.00 ^{nopqrstu} (0.45)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^v (0.23)	16.00 ^{opqrstuv} (0.41)	52.00 ^{ghi} (0.81)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^v (0.23)	32.00 ^{klmno} (0.59)	76.00 ^{def} (1.06)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
5	10	0.00 ^v (0.23)	0.00 ^v (0.23)	0.00 ^v (0.23)	12.00 ^{qrstuv} (0.37)	0.00 ^v (0.23)	12.00 ^{qrstuv} (0.37)
	20	0.00 ^v (0.23)	0.00 ^v (0.23)	0.00 ^v (0.23)	44.00 ^{ijkl} (0.72)	80.00 ^{bcdef} (1.11)	84.00 ^{bcde} (1.16)
	30	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	8.00 ^{tuv} (0.32)	64.00 ^{fgh} (0.94)	88.00 ^{abcd} (1.21)	92.00 ^{abc} (1.25)
	40	0.00 ^v (0.23)	0.00 ^v (0.23)	32.00 ^{klmnop} (0.58)	76.00 ^{cdef} (1.07)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	48.00 ^{hij} (0.77)	80.00 ^{bcdef} (1.12)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^v (0.23)	4.00 ^{uv} (0.27)	68.00 ^{efg} (0.98)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 15. Effect of power of microwave radiation on pupae of *Tribolium castaneum*

Power (W)	Mortality of pupa (%)
Control	0.00 ^f (0.23)
136	27.60 ^d (0.53)
264	50.13 ^c (0.79)
440	66.40 ^b (0.97)
616	82.27 ^a (1.15)
800	84.67 ^a (1.17)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 16. Effect of flour bed thickness on pupae of *Tribolium castaneum*

Flour bed thickness (cm)	Mortality of pupa (%)
1	66.22 ^a (0.97)
2	61.78 ^b (0.92)
3	50.11 ^c (0.77)
4	44.89 ^d (0.73)
5	36.22 ^e (0.63)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 17. Effect of microwave exposure time on pupae of *Tribolium castaneum*

Exposure time (s)	Mortality of pupa (%)
10	19.07 ^e (0.44)
20	41.20 ^d (0.69)
30	52.40 ^c (0.81)
40	59.07 ^b (0.89)
50	68.53 ^a (0.99)
60	70.80 ^a (1.02)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

At the lowest irradiation dose (136 W), even though pupal mortality decreased with an increase in flour bed thickness from four cm (10.67 per cent) to five cm (4.67 per cent), the values were comparable. But, at the remaining higher doses, mortality significantly differed between four cm and five cm. All the pupae released in control survived and was comparable with mortality at 136 W and five cm flour bed thickness.

4.3.5 Interaction effect of power and time on pupae of *Tribolium castaneum*

The impact of microwave power and exposure time on pupae of *T. castaneum* indicates that the highest pupal mortality occurred with an increase in both power and time (Table 19). Thus complete death (100.00 per cent) of released pupae occurred at 800 W treated for 50 and 60 s and at 616 W, with 60 s exposure time. Pupal mortality in samples irradiated at 616 W for 30 to 50 s, at 800 W for 30 and 40 s and 440 W for 50 and 60 s was on par among each other and also with treatments that produced complete mortality.

The highest pupal mortality recorded at 264 W for the longest exposure time (60 s) was 86.40 per cent, and it did not differ significantly with mortality at 30 s when the power of microwave radiation was 616 W (92.00 per cent) and 800 W (93.60 per cent). The former showed comparable mortality when irradiated at 440 and 800 W, respectively, for 40 and 20 s.

Pupal mortality at 440 W statistically differed between irradiation times from 10 to 40 s, but mortality was comparable at 50 and 60 s exposure time. At 264 W, mortality was significantly higher at 60 s exposure, but mortality at 20 (33.60 per cent) and 30 s (40.80 per cent) were on par.

Pupal mortality at the lowest exposure (10 s) and highest power (616, 800 W) was on par with mortality at the lowest dose (136 W) and at the two highest exposure times (50, 60 s). At 136 W, even though we increased the exposure time from 30 to 40 s, mortality was comparable, and the same trend was similar between 10 and 20 s. Pupae exposed to microwave radiation for the shortest time of 10 s showed comparable mortalities irrespective of the increase in power from 136 to 440 W. All the pupae in control developed to adults and was significantly different at all the treatment combinations.

Table 18. Interaction effect of power and thickness on pupae of *Tribolium castaneum*

Power (W)	Thickness (cm)				
	1	2	3	4	5
Control	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)
136	40.67 ^{hi} (0.68)	46.00 ^h (0.74)	36.00 ⁱ (0.62)	10.67 ^k (0.34)	4.67 ^{kl} (0.28)
264	75.33 ^{cde} (1.07)	68.67 ^{ef} (0.99)	43.33 ^{hi} (0.71)	38.00 ^{hi} (0.65)	25.33 ^j (0.51)
440	88.00 ^b (1.21)	78.67 ^{cd} (1.10)	58.00 ^g (0.88)	61.33 ^{fg} (0.91)	46.00 ^h (0.74)
616	96.67 ^a (1.31)	88.67 ^{ab} (1.22)	80.67 ^{bc} (1.13)	76.67 ^{cd} (1.09)	68.67 ^{ef} (0.99)
800	96.67 ^a (1.31)	88.67 ^{ab} (1.22)	82.67 ^{bc} (1.15)	82.67 ^{bc} (1.15)	72.67 ^{de} (1.04)

*Figures in the parentheses are arc sine transformed values
Values with the same letters are not significantly different

Table 19. Interaction effect of power and time on pupae of *Tribolium castaneum*

Power (W)	Time (s)					
	10	20	30	40	50	60
Control	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)	0.00 ⁿ (0.23)
136	14.40 ^{lm} (0.39)	18.40 ^{lm} (0.44)	23.20 ^{kl} (0.49)	31.20 ^{jk} (0.58)	39.20 ^{hij} (0.65)	39.20 ^{hij} (0.66)
264	12.00 ^m (0.36)	33.60 ^{ij} (0.60)	40.80 ^{hi} (0.69)	50.40 ^g (0.80)	77.60 ^{de} (1.09)	86.40 ^{bc} (1.19)
440	16.00 ^{lm} (0.41)	43.20 ^{gh} (0.71)	64.80 ^f (0.96)	79.20 ^{cd} (1.11)	96.00 ^a (1.30)	99.20 ^a (1.34)
616	38.40 ^{hij} (0.66)	69.60 ^{ef} (1.00)	92.00 ^{ab} (1.25)	95.20 ^a (1.29)	98.40 ^a (1.33)	100.00 ^a (1.35)
800	33.60 ^{ij} (0.61)	82.40 ^{cd} (1.14)	93.60 ^{ab} (1.27)	98.40 ^a (1.33)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values
Values with the same letters are not significantly different at 5.00% level by LSD

4.3.6 Interaction effect of thickness and time on pupae of *Tribolium castaneum*

Upon the analysis on the effect of the thickness-time combination on mortality of pupae of *T. castaneum* (Table 20), it could be noted that, at the lower flour thickness (one cm) and longest treatment time (60 s), pupal mortality was 78.67 per cent. The data indicate that pupal mortality increased at each flour bed thickness with a rise in time of exposure. At one cm flour bed thickness, and at 40 and 50 s time period, the pupal mortality was 72.00 and 77.33 per cent, respectively, which was on par with the highest recorded mortality. They were also comparable with mortality at flour bed thickness of two cm exposed for 40 to 60 s and three cm thickness treated for 60 s.

Pupal mortality at 50 and 60 s at each flour bed thickness did not differ statistically from each other. At one cm, mortality at 20 s (62.00 per cent) and 30 s (69.33 per cent) were comparable, and the latter was comparable with mortality at 40 s (72.00 per cent). At two cm, there was comparable mortality between two exposure times, *i.e.*, at 30 (68.00 per cent) and 40 s (70.67 per cent), but differed significantly from mortality at lower exposure period. At flour bed thickness of three cm and four cm, pupal death differed significantly between all treatment times from 10 to 40 s. At five cm, the per cent inhibition of pupal development was comparable between 20 (23.33 per cent) and 30 s (28.00 per cent).

The pupal mortality at one cm flour bed thickness, at 10 s exposure time was 38.00 per cent, which was the lowest compared to mortalities at higher exposure. Similarly, at other flour bed thicknesses also, the mortality was lowest at 10 s. Thus, a longer microwave exposure time is required to attain the death of more pupae. Mortality of pupae at higher flour bed thicknesses of three, four and five cm, irradiated for 10 s were 11.33, 10.67 and 8.00 per cent respectively, which were comparable and the lowest observed mortality in the interaction effect of thickness and time on pupae of *T. castaneum*.

Table 20. Interaction effect of thickness and time on pupae of *Tribolium castaneum*

Thickness (cm)	Time (s)					
	10	20	30	40	50	60
1	38.00 ^k (0.65)	62.00 ^{efgh} (0.92)	69.33 ^{bcde} (1.00)	72.00 ^{abc} (1.03)	77.33 ^{ab} (1.09)	78.67 ^a (1.12)
2	27.33 ^l (0.54)	55.33 ^{hi} (0.84)	68.00 ^{cdef} (0.98)	70.67 ^{abcd} (1.02)	74.00 ^{abc} (1.05)	75.33 ^{abc} (1.07)
3	11.33 ^m (0.36)	38.00 ^k (0.65)	51.33 ^{ij} (0.80)	60.00 ^{fgh} (0.90)	68.67 ^{cde} (0.99)	71.33 ^{abcd} (1.01)
4	10.67 ^m (0.35)	27.33 ^l (0.54)	45.33 ^{jk} (0.74)	54.67 ^{hi} (0.84)	64.00 ^{defg} (0.93)	67.33 ^{cdef} (0.98)
5	8.00 ^m (0.32)	23.33 ^l (0.49)	28.00 ^l (0.54)	38.00 ^k (0.65)	58.67 ^{ghi} (0.88)	61.33 ^{efgh} (0.91)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

4.3.7 Interaction effect of power, thickness and time on pupae of *Tribolium castaneum*

The interaction of power, thickness and time reveals that, pupal mortality increases as we increase the power, reduce the thickness and increase the exposure period of microwave radiation (Table 21). At a flour thickness of one cm, 100 per cent mortality could be observed with 20 s exposure at 616 and 800 W. As the exposure time increased to 30 s, 100 per cent death of pupae occurred at a lower dose of 440 W. Though at 264 W, complete pupal mortality was not achieved at any of the treatment periods, mortality at 30 (88.00 per cent), 40 (92.00 per cent), 50 (92.00 per cent) and 60 s (96.00 per cent) exposure was statistically comparable with cent per cent mortality attained at the higher doses. Per cent mortality at the lowest dose of 136 W, at the longest exposure time of 60 s was 76.00 per cent, which differed significantly with the highest mortality. Exposing pupae at doses of 136 and 264 W, for 10 s exposure, did not result in mortality statistically higher than mortality in control (0.00 per cent).

The irradiated pupae at two cm flour thickness at 440 W or at higher doses, died from 30 s onwards. There was 96.00 per cent mortality of pupae at 800 W, at 20 s exposure time and was on par with cent per cent mortality. At 264 W, pupal mortality increased from 44.00 to 96.00 per cent as the exposure time increased from 10 to 60 s. The mortality of 88.00 and 96.00 per cent at 50 and 60 s exposure, respectively at 264 W, was also comparable to cent per cent mortality. At 136 W, only 56.00 per cent of pupae died even at the highest exposure time of 60 s and were significantly superior over control.

There was complete mortality of pupae at three cm flour thickness at 616 and 800 W, when treatment time was 30 s or above. Even at 440 W, the increase of irradiation period to 50 s resulted in complete death of all the released pupae. Pupal mortality at 616 and 800 W, both at 20 s and at 440 at 40 s, was similar, *i.e.*, 80.00 per cent. Exposing pupae at 264 W for 60 s caused death of 88.00 per cent, comparable with the highest mortality. The death rate of pupae at the lowest dose (136 W) was

comparable to each other at all exposure times. At the same time, the pupal mortality at 264 and 440 W, at 10 s was comparable with mortality in control.

At the highest power of 800 W, pupal mortality was 100.00 per cent when treated for 30 s or above, at a flour bed thickness of four cm. Reducing the microwave power to 616 W and 440 W resulted in the complete death of pupae only after increasing the exposure time to 50 s and 60 s, respectively. There was comparable pupal mortality at 616 W, at 30 (96.00 per cent) and 40 s (96.00 per cent), at 440 W at 40 (84.00 per cent) and 50 s (96.00 per cent) and at 264 W at 60 s (84.00 per cent) exposure. In control, all the released pupae emerged to adults. Mortality of pupae observed at 136 W when irradiated for 10 to 50 s was not significantly higher than mortality in control. Even though we increased the microwave power from 136 W to 440 W, pupal mortality was on par with control by keeping the exposure time as 10 s.

At the highest flour thickness of five cm, there was complete pupal mortality only at higher power of 800 W, irradiated for 50 and 60 s and at 616 W, when treated for 60 s. Pupal mortality at 440, 616 and 800 W, at 50 (84.00 per cent) and 60 s (96.00 per cent), 50 s (92.00 per cent), and 40 s (92.00 per cent), respectively, was on par with the highest mortality (100.00 per cent). Mortality at 136 W at all exposure times, at 264 W, at shorter exposure periods (10 to 40 s) and at 264 at 10 s exposure was comparable with mortality in control (0.00 per cent).

A line of conclusion says that irradiation at 440 W was sufficient to cause total mortality of pupae of *T. castaneum* up to four cm flour thickness. Exposure for 30 s was sufficient to cause complete mortality up to two cm flour thickness. But, increasing the bed thickness to three and four cm required to raise the exposure to 50 and 60 s, respectively to obtain cent per cent mortality, and even at 60 s exposure, 440 W was not sufficient to cause total mortality of pupae at five cm flour bed thickness. Irradiating at 616 W or above cause cent per cent mortality with 20 s exposure at one cm thickness and 50 s exposure at four cm flour bed thickness. At a flour bed thickness of five cm, an exposure of 60 s only resulted in 100 per cent mortality at 616 W. At the highest power of 800 W, cent per cent mortality occurred with 20 s

exposure at one cm, 30 s exposure at two, three and four cm and 50 s exposure or more at the highest flour bed thickness of five cm.

4.4 EFFECT OF MICROWAVE RADIATION ON ADULTS OF *Tribolium castaneum*

4.4.1 Effect of power of microwave radiation on 17±2 day old adults of *Tribolium castaneum*

Exposure of 17±2 day old adults of red flour beetle to microwave radiation ranging from 136 W to 800 W helped to study the impact of irradiation on mortality of beetles (Table 22). The highest power of 800 W resulted in significantly higher adult mortality of 86.27 per cent over all other doses. At 616 W, mortality was 80.13 per cent which was statistically superior over all the other lower doses. There was no mortality of adults in control. The adult mortality at 264 W and 440 W were 45.60 per cent and 68.67 per cent, respectively, which differed significantly among each other and from other treatments. At the lowest dose of 136 W, there was significantly higher mortality of 10.80 per cent over control but significantly lower to all the other treatments (Plate 14).

4.4.2 Effect of flour bed thickness on 17±2 day old adults of *Tribolium castaneum*

Mortality of 17±2 day old adults of *T. castaneum* decreased significantly from 64.22 to 37.11 per cent with an increase in flour bed thickness from one cm to five cm, which shows that adult mortality and flour bed thickness are inversely proportional to each other (Table 23). Mortality at two cm was 52.11 per cent which is statistically superior to mortalities at higher thicknesses and is the next best treatment after at one cm bed thickness. The per cent mortality observed at three and four cm flour bed thickness was 48.44 and 41.00 per cent, respectively, and they differed significantly among each other and with all other treatments.

Table 21. Interaction effect of power, thickness and time on pupae of *Tribolium castaneum*

Thickness (cm)	Time (s)	Power (W)					
		Control	136	264	440	616	800
1	10	0.00 ^E (0.23)	8.00 ^{CDE} (0.32)	4.00 ^{DE} (0.27)	56.00 ^{ijklmnopq} (0.85)	80.00 ^{bcdefg} (1.12)	80.00 ^{bcdefg} (1.11)
	20	0.00 ^E (0.23)	20.00 ^{vwxyzABCD} (0.46)	80.00 ^{bcdefg} (1.11)	72.00 ^{defghijk} (1.03)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^E (0.23)	28.00 ^{tuvwxyzAB} (0.55)	88.00 ^{abcde} (1.20)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^E (0.23)	40.00 ^{pqrstuvw} (0.67)	92.00 ^{abcd} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^E (0.23)	72.00 ^{efghijk} (1.02)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^E (0.23)	76.00 ^{bcdefgh} (1.08)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
2	10	0.00 ^E (0.23)	28.00 ^{tuvwxyzA} (0.55)	44.00 ^{nopqrstu} (0.71)	44.00 ^{nopqrstu} (0.71)	56.00 ^{hijklmnop} (0.85)	36.00 ^{pqrstuvw} (0.64)
	20	0.00 ^E (0.23)	36.00 ^{qrstuvwxy} (0.63)	52.00 ^{klmnopqrs} (0.80)	72.00 ^{efghijk} (1.02)	76.00 ^{bcdefghi} (1.07)	96.00 ^{ab} (1.30)
	30	0.00 ^E (0.23)	48.00 ^{mnpqrst} (0.76)	60.00 ^{ghijklmno} (0.90)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^E (0.23)	52.00 ^{ijklmnopqrs} (0.81)	72.00 ^{defghijk} (1.03)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^E (0.23)	56.00 ^{ijklmnopq} (0.85)	88.00 ^{abcde} (1.20)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^E (0.23)	56.00 ^{ijklmnopq} (0.85)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
3	10	0.00 ^E (0.23)	16.00 ^{xyzABCDE} (0.42)	12.00 ^{zABCDE} (0.37)	8.00 ^{BCDE} (0.32)	24.00 ^{vwxyzABC} (0.50)	32.00 ^{stuvwxyz} (0.59)
	20	0.00 ^E (0.23)	32.00 ^{stuvwxy} (0.60)	24.00 ^{vwxyzABC} (0.50)	12.00 ^{zABCDE} (0.37)	80.00 ^{bcdefg} (1.11)	80.00 ^{bcdefg} (1.11)
	30	0.00 ^E (0.23)	36.00 ^{rstuvwxy} (0.62)	24.00 ^{vwxyzABC} (0.50)	48.00 ^{lmnopqrst} (0.77)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^E (0.23)	40.00 ^{opqrstuv} (0.68)	40.00 ^{pqrstuvw} (0.67)	80.00 ^{bcdefg} (1.11)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^E (0.23)	40.00 ^{pqrstuvw} (0.67)	72.00 ^{efghijk} (1.02)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^E (0.23)	40.00 ^{stuvwxy} (0.62)	88.00 ^{abcde} (1.21)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

4	10	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	32.00 ^{stuvwxyz} (0.59)	24.00 ^{uvwxyzABC} (0.50)
	20	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	12.00 ^{ABCDE} (0.3)	40.00 ^{opqrstu} (0.69)	36.00 ^{pqrstuvw} (0.64)	72.00 ^{cdefghij} (1.03)
	30	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	28.00 ^{tuvwxyza} (0.55)	44.00 ^{nopqrstu} (0.73)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	40	0.00 ^E (0.23)	16.00 ^{yzABCDE} (0.41)	32.00 ^{stuvwxy} (0.60)	84.00 ^{abcdef} (1.16)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	50	0.00 ^E (0.23)	16.00 ^{ABCDE} (0.36)	72.00 ^{cdefghij} (1.03)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^E (0.23)	20.00 ^{vwxzyABCD} (0.46)	84.00 ^{abcdef} (1.16)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
5	10	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	0.00 ^E (0.23)	12.00 ^{zABCDE} (0.37)	20.00 ^{wxyzABCD} (0.45)	20.00 ^{wxyzABCD} (0.45)
	20	0.00 ^E (0.23)	0.00 ^E (0.23)	0.00 ^E (0.23)	20.00 ^{vwxzyAB} CD (0.46)	56.00 ^{klmnopqr} (0.84)	64.00 ^{ghijklmn} (0.93)
	30	0.00 ^E (0.23)	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	32.00 ^{stuvwxyz} (0.59)	64.00 ^{ghijklmn} (0.93)	68.00 ^{ghijklm} (0.98)
	40	0.00 ^E (0.23)	8.00 ^{BCDE} (0.32)	16.00 ^{xyzABCDE} (0.41)	32.00 ^{stuvwxyz} (0.59)	80.00 ^{bcdefg} (1.11)	92.00 ^{abcd} (1.25)
	50	0.00 ^E (0.23)	12.00 ^{ABCDE} (0.36)	64.00 ^{ghijklmn} (0.93)	84.00 ^{abcdef} (1.16)	92.00 ^{abcd} (1.25)	100.00 ^a (1.35)
	60	0.00 ^E (0.23)	4.00 ^{DE} (0.27)	68.00 ^{efghijkl} (0.99)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 22. Effect of power of microwave radiation on 17±2 day old adults of *Tribolium castaneum*

Power (W)	Adult mortality (%)
Control	0.00 ^f (0.23)
136	10.80 ^e (0.35)
264	45.60 ^d (0.74)
440	68.67 ^c (0.99)
616	80.13 ^b (1.12)
800	86.27 ^a (1.19)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 23. Effect of flour bed thickness on 17±2 day old adults of *Tribolium castaneum*

Flour bed thickness (cm)	Adult mortality (%)
1	64.22 ^a (0.95)
2	52.11 ^b (0.81)
3	48.44 ^c (0.77)
4	41.00 ^d (0.69)
5	37.11 ^e (0.64)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 24. Effect of microwave exposure time on 17±2 day old adults of *Tribolium castaneum*

Exposure time (s)	Adult mortality (%)
10	17.60 ^f (0.43)
20	42.53 ^e (0.70)
30	50.00 ^d (0.78)
40	55.60 ^c (0.85)
50	60.13 ^b (0.90)
60	65.60 ^a (0.96)

*Figures in the parentheses are arc sine transformed values

Values with same letters are not significantly different at 5.00% level by LSD

4.4.3 Effect of microwave exposure time on 17±2 day old adults of *Tribolium castaneum*

The study on the impact of microwave exposure time on the mortality of 17±2 day old adults of *T. castaneum* revealed that exposure time has a positive effect on mortality (Table 24). The mortality of adults at 10 s was 17.60 per cent, significantly different and lower than other treatments. At 20, 30, 40 and 50 s exposure time, per cent mortality recorded was 42.53, 50.00, 55.60 and 60.13 per cent, respectively, and they statistically differed. The best exposure time at which maximum mortality (65.60 per cent) recorded was 60 s.

4.4.4 Interaction effect of power and thickness on 17±2 day old adults of *Tribolium castaneum*

At the highest power (800 W) and lower flour bed thickness (one cm), maximum mortality of adults recorded was 98.67 per cent (Table 25). It was comparable with mortality at 616 W, one cm flour bed thickness (92.00 per cent) and 800 W, at two (94.67 per cent) and three cm (92.00 per cent) flour bed thickness. The adult mortality did not differ significantly between four and five cm at irradiation power of 800 W. A similar trend could be observed at 440 W in a flour bed thickness of four and five cm. At 616 W, mortality at one cm and two cm (84.00 per cent) were on par and the latter in turn is comparable with mortality at three (80.67 per cent) and four cm (78.67 per cent) thickness. At 440 W, adult mortality at one cm (87.33 per cent) and two cm (80.00 per cent) flour thickness did not differ statistically. At 264 W, mortality at three thicknesses, *i.e.*, at two, three and four cm, were on par with each other but differed significantly with mortality at the highest and lower flour bed thickness.

At the lowest irradiation dose of 136 W, adult mortality was comparatively lower when compared to mortalities at the higher doses. At one cm flour bed thickness, 31.33 per cent of adults couldn't survive microwave radiation, but when the thickness of flour bed increased to five cm, the mortality significantly reduced to 1.33 per cent. Adult mortality at three, four, and five cm were 6.67, 4.00 and 1.33 per cent, respectively. They were comparable to mortality in control (0.00 per cent), which

means adults of red flour beetles are relatively unaffected by irradiation at 136 W, at higher flour bed thickness.

The highest mortality attained at five cm flour bed thickness was at 800 W (74.67 per cent), comparable with mortality at 616, 440 and 264 W at three and four cm, two cm and one cm flour bed thickness, respectively. Hence, higher doses can inhibit the adult survival even at higher flour bed thickness. At the same time, at lower power levels, comparable mortality could be achieved by reducing the flour bed thickness.

4.4.5 Interaction effect of power and time on 17±2 day old adults of *Tribolium castaneum*

Microwave radiation completely arrested the growth and development of 17±2 day old adults of red flour beetle, *i.e.*, the mortality was 100.00 per cent at the highest dose of 800 W when exposed for more extended periods (50 and 60 s) (Table 26). At relatively lower treatment times of 30 and 40 s and at 800 W power, mortality was appreciably high, *i.e.*, 91.20 and 93.60 per cent, respectively, and was comparable to treatments with complete mortality. At the same dose but at shorter exposure times of 10 and 20 s, mortality was significantly lower compared to longer treatment times.

The wheat flour containing adults irradiated at 616 W for 50 and 60 s caused 97.60 per cent mortality, comparable to mortality at the same dose, treated for 40 s (96.80 per cent). Similarly, at 440 W and 264 W, adult mortality at 50 and 60 s were comparable. Per cent mortality of adults at 264 W increased from 8.80 to 79.20, with the rise in exposure time from 10 to 60 s. Adult mortality with 20 (28.80 per cent), 30 (40.00 per cent), and 40 s (57.60 per cent) exposure differed significantly between each other. However, mortality did not increase significantly by increasing exposure time from 40 s to 50 s (59.20 per cent).

Irradiation of adults of *T. castaneum* at 136 W for 10 s was not sufficient to cause any mortality, similar to control. Even though we increased the microwave exposure time to 20 s, at 136 W, mortality was very low (6.40 per cent) and comparable with control. Even at the highest treatment time, mortality was

Table 25. Interaction effect of power and thickness on 17±2 day old adults of *Tribolium castaneum*

Power (W)	Thickness (cm)				
	1	2	3	4	5
Control	0.00 ^p (0.23)	0.00 ^p (0.23)	0.00 ^p (0.23)	0.00 ^p (0.23)	0.00 ^p (0.23)
136	31.33 ^{mn} (0.58)	10.67 ^o (0.35)	6.67 ^{op} (0.30)	4.00 ^{op} (0.27)	1.33 ^p (0.24)
264	76.00 ^{efg} (1.07)	43.33 ^l (0.71)	45.33 ^{kl} (0.73)	39.33 ^{lm} (0.67)	24.00 ⁿ (0.50)
440	87.33 ^{bcd} (1.21)	80.00 ^{def} (1.12)	66.00 ^h (0.96)	52.67 ^{jk} (0.82)	57.33 ^{ij} (0.87)
616	92.00 ^{abc} (1.26)	84.00 ^{cde} (1.17)	80.67 ^{def} (1.13)	78.67 ^{efg} (1.10)	65.33 ^{hi} (0.96)
800	98.67 ^a (1.33)	94.67 ^{ab} (1.28)	92.00 ^{abc} (1.26)	71.33 ^{gh} (1.03)	74.67 ^{fg} (1.06)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 26. Interaction effect of power and time on 17±2 day old adults of *Tribolium castaneum*

Power (W)	Time (s)					
	10	20	30	40	50	60
Control	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)	0.00 ^l (0.23)
136	0.00 ^l (0.23)	6.40 ^{kl} (0.30)	10.40 ^{jk} (0.34)	8.80 ^{jkl} (0.33)	16.00 ^{ij} (0.41)	23.20 ^{hi} (0.49)
264	8.80 ^{jk} (0.33)	28.80 ^h (0.55)	40.00 ^g (0.67)	57.60 ^f (0.87)	59.20 ^f (0.89)	79.20 ^{dc} (1.11)
440	13.60 ^{jk} (0.38)	64.00 ^f (0.94)	76.00 ^e (1.08)	76.80 ^e (1.08)	88.00 ^{bcd} (1.21)	93.60 ^{ab} (1.27)
616	28.00 ^h (0.55)	78.40 ^e (1.10)	82.40 ^{cde} (1.14)	96.80 ^{ab} (1.31)	97.60 ^a (1.32)	97.60 ^a (1.32)
800	55.20 ^f (0.85)	77.60 ^e (1.10)	91.20 ^{abc} (1.25)	93.60 ^{ab} (1.27)	100.00 ^a (1.35)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

only 23.20 per cent, which did not significantly differ from mortality with 50 s exposure (16.00 per cent).

4.4.6 Interaction effect of thickness and time on 17±2 day old adults of *Tribolium castaneum*

The comparison of the interaction of thickness and time, shows that, the mortality of adults increased when we reduced the thickness and increased the exposure time (Table 27). So at the lowest flour thickness (one cm) and at longer treatment times of 40, 50 and 60 s, adult mortality was highest, *i.e.*, 72.00, 73.33 and 74.00 per cent, respectively. Even the mortalities at relatively shorter exposure times of 20 (66.67 per cent) and 30 s (68.00 per cent) were comparable to this.

At two cm flour thickness, adult mortality at 10 s (22.00 per cent) was significantly lower from other treatment times. However, mortalities at subsequent exposure time from 20 (48.00 per cent) and 30 s (53.33 per cent) were comparable to one another. The mortality with 30 s exposure was comparable to that with mortality at 40 s (58.00 per cent). Adult mortality at 50 (62.67 per cent) and 60 s (68.67 per cent) did not differ significantly. The same trend could be observed at three cm and five cm flour bed thickness, where the mortality at 10 s was substantially lower than all other exposure times. But mortality at 20 s and 30 s; 30 s and 40 s; 40 s and 50 s and 50 s and 60 s did not differ significantly.

At four cm flour bed thickness, mortality of adults at 10 (8.00 per cent), 20 (25.33 per cent) and 30 s (42.67 per cent) were significantly different. But, per cent mortality at 40 s (52.00 per cent) was comparable with 50 s (56.00 per cent); the latter, in turn, did not differ statistically from mortality at 60 s (62.00 per cent). Only 6.00 per cent of adults died at the thickness-time combination of 5 cm and 10 s and were the worst treatment combination.

A significant reduction in adult mortality can be observed with the increasing flour thickness at each period. For example, at 20 s exposure, the mortality almost halved as we raised the flour thickness from one (66.67 per cent) to five cm (32.00 per cent).

Table 27. Interaction effect of thickness and time on 17±2 day old adults of *Tribolium castaneum*

Thickness (cm)	Time (s)					
	10	20	30	40	50	60
1	31.33 ^{kl} (0.58)	66.67 ^{abc} (0.97)	68.00 ^{abc} (0.98)	72.00 ^a (1.03)	73.33 ^a (1.05)	74.00 ^a (1.05)
2	22.00 ^m (0.48)	48.00 ^{hi} (0.76)	53.33 ^{fgh} (0.82)	58.00 ^{def} (0.88)	62.67 ^{bcd} (0.93)	68.67 ^{ab} (0.99)
3	20.67 ^m (0.46)	40.67 ^{ij} (0.68)	48.67 ^{ghi} (0.77)	54.00 ^{efgh} (0.83)	60.00 ^{cdef} (0.90)	66.67 ^{abc} (0.97)
4	8.00 ⁿ (0.32)	25.33 ^{lm} (0.51)	42.67 ^{ij} (0.70)	52.00 ^{fgh} (0.81)	56.00 ^{defgh} (0.85)	62.00 ^{bcde} (0.92)
5	6.00 ⁿ (0.30)	32.00 ^{kl} (0.58)	37.33 ^{jk} (0.64)	42.00 ^{ij} (0.70)	48.67 ^{ghi} (0.77)	56.67 ^{defg} (0.86)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Per cent mortality of adults at a flour thickness of one cm and at 10 s exposure (31.33 per cent) was comparable with mortality at five cm flour bed thickness, both at 20 (32.00 per cent) and 30 s (37.33 per cent) exposure. Hence, it is clear that mortality attained at lower flour thickness could be achieved even at higher flour thickness if we increase the irradiation time.

4.4.7 Interaction effect of power, thickness and time on 17±2 day old adults of *Tribolium castaneum*

By maintaining the bed thickness at one cm and irradiating the adults of *T. castaneum* at 440, 616 and 800 W resulted in complete mortality even from an exposure time of 20 s (Table 28). Microwave heating at 264 and 136 W was not sufficient to obtain total adult mortality. But, adult mortality at 264 W, when treated for 40 (92.00 per cent), 50 (96.00 per cent), and 60 s (96.00 per cent), were comparable with the complete mortality obtained at the higher doses of irradiation. At 800 W, even at the lowest exposure time (10 s), there was 92.00 per cent mortality of adults, comparable with cent per cent mortality. At the lowest power of 136 W, only 48.00 per cent of adults died even at the longest exposure time of 60 s. Adult mortality at 136 W at 10 s exposure did not differ statistically from 0.00 per cent mortality in control.

At two cm flour thickness, complete death of adults occurred at 800 W when irradiated from 30 s onwards. At a relatively lower dose of 616 W, a cent per cent mortality of adults occurred when we raised the exposure time to 40 s. While at 440 W, complete death of adults could be observed only after increasing the exposure time to 50 s. The mortality of adults at 264 W with 60 s exposure was 84.00 per cent. It was on par with mortality at 440 W with 20 (84.00 per cent), 30 (88.00 per cent) and 40 s (88.00 per cent) exposure, at 616 W with 20 (88.00 per cent) and 30 s (88.00 per cent) exposure and at 800 W treated for 20 s (96.00 per cent), which was also comparable to cent per cent mortality. Adult mortality at 136 W, at all treatment times except 60 s was comparable with mortality in control.

The increase in flour bed thickness to three cm and adults irradiated at 800 W, there was a cent per cent mortality at 40 s. When the microwave power lowered to 616

and 440 W, complete death occurred only after raising the treatment time to 50 and 60 s, respectively. Complete mortality obtained at various doses and exposure time was comparable to mortality at 440 W with 50 s exposure (88.00 per cent), 616 W with 30 (88.00 per cent) and 40 s (96.00 per cent) and 800 W with 30 s (96.00 per cent) exposure. Irradiation at 264 and 136 W was not sufficient to attain cent per cent mortality. Adult mortality at 264 W, even at the highest exposure of 60 s (80.00 per cent), was significantly different from cent per cent mortality. All the adults survived in control, comparable with adult mortality at 136 W, from 10 to 50 s time period.

Adult mortality at four cm flour bed thickness reached a maximum of 100.00 per cent only at the highest dose of 800 W when irradiated for 50 and 60 s. There was 84.00 per cent mortality of adults at 616 and 800 W, at 20 s and 440 W, at 50 s, which were comparable to the cent per cent mortality. Similarly, 88.00 per cent adult mortality at 800 W with 40 s exposure, 92.00 per cent mortality at 440 and 616 W at 60 s exposure and 96.00 per cent mortality at 616 W at 40 and 50 s exposure was also comparable with complete mortality. At a microwave power of 264 W, at 60 s treatment time, adult mortality was 76.00 per cent, which differed statistically from cent per cent mortality. At the lowest power of 136 W, even though we exposed the adults for 50 and 60 s, the mortality was 12.00 per cent, which was on par with 0.00 per cent mortality observed at lower treatment times (10 to 40 s) and control.

At a maximum flour thickness of five cm, irradiating at 800 W for 50 and 60 s killed all the released adults of red flour beetle. At 616 W, at longer exposure times of 40, 50 and 60 s, adult mortality was 92.00, 92.00 and 96.00 per cent, respectively, comparable with 100.00 per cent mortality observed at the highest dose. Mortality of adults at 264 and 440 W, at the highest exposure time of 60 s was 60.00 and 76.00 per cent, respectively. They did not differ significantly among each other but differed from the highest mortality of 100.00 per cent. Irradiation at 136 W for 10 to 50 s and at 264 W for 10, and 20 s was not sufficient to kill the released adults. At 136 W, even at 60 s, adult mortality was only 8.00 per cent, and they all were comparable with 0.00 per cent mortality observed in control.

To conclude, a power of 440 W was sufficient to cause cent per cent mortality of adults even with 20 s exposure at one cm flour bed thickness, while a higher exposure of 50 and 60 s only caused total mortality at two and three cm flour thickness. Increasing the flour thickness above three cm did not cause complete mortality of adults of *T. castaneum* at 440 W. At 616 W, an exposure of 40 s resulted in 100 per cent mortality of adults at two cm flour thickness and 50 s at three cm flour bed thickness. Increasing the flour thickness over three cm did not result in total mortality of adults at 616 W. At 800 W, 50 s exposure was sufficient to cause 100 per cent mortality of adults at four and five cm flour thickness, and reducing the flour thickness to one cm resulted in total mortality of adults with 20 s exposure, two cm at 30 s exposure and three cm at 40 s exposure.

4.5 EFFECT OF MICROWAVE RADIATION ON NUTRITIONAL FACTORS OF WHEAT FLOUR

Microwave treatment at 800 W for 50 s, at higher flour bed thicknesses of four (T4) and five cm (T5), gave complete mortality of all the developmental stages of *T. castaneum*. Therefore, they were considered the best treatment combinations in the irradiation and selected to analyse various quality parameters.

4.5.1 Effect of microwave radiation on moisture, gluten and vitamin B content of wheat flour

The moisture content of wheat flour samples irradiated at 800 W power level for 50 s was compared by keeping the samples at four cm and five cm flour thickness with untreated control (Table 29). Moisture content in control was 10.63 per cent, while it reduced to 7.95 per cent and 9.43 per cent in T4 and T5, respectively. The result suggested that the moisture content of wheat flour decreased significantly with microwave irradiation.

The dry gluten content of wheat flour samples irradiated at 800 W for 50 s at four cm flour thickness was 7.15 per cent and was statistically comparable with gluten content in control (7.95 per cent) (Table 29). Gluten content at the same power-time combination with flour thickness of five cm was 7.84 per cent, which was also on par

with control. Thus, microwave irradiation did not influence the gluten content of wheat flour.

The content of vitamin B₁, B₂ and B₆ slightly increased after microwave irradiation, whereas B₃ content decreased (Table 29). Vitamin B₁ content was 11.90 ppm in control, which increased to 12.45 ppm in T4 and 12.70 ppm in T5. Vitamin B₂ also slightly increased from 0.03 ppm in control to 0.07 and 0.04 ppm in T4 and T5, respectively. Vitamin B₆ content increased to 6.98 ppm in T4 and 7.24 ppm in T5 from 4.28 ppm in control. Vitamin B₃ content decreased from 14.17 ppm in control to 11.63 ppm and 12.42 ppm after microwave irradiation in T4 and T5, respectively. Vitamin B₅ was not detected in both treated as well as control samples.

4.5.2 Effect of microwave radiation on colour of wheat flour

We estimated the colour change of wheat flour samples in control and the two treatment samples (Table 30). The estimated colour change of irradiated wheat flour samples (at 800 W for 50 s) at four cm (0.64) and five cm (0.41) did not differ significantly from control.

Table 28. Interaction effect of power, thickness and time on 17±2 day old adults of *Tribolium castaneum*

Thickness (cm)	Time (s)	Power (W)					
		Control	136	264	440	616	800
1	10	0.00 ^z (0.23)	0.00 ^z (0.23)	20.00 ^{uvwxy} (0.46)	24.00 ^{tuvwx} (0.50)	52.00 ^{ijklmnop} (0.81)	92.00 ^{abc} (1.25)
	20	0.00 ^z (0.23)	24.00 ^{uvwxy} (0.49)	76.00 ^{bcdefghi} (1.07)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	30	0.00 ^z (0.23)	32.00 ^{pqrstuvw} (0.59)	76.00 ^{bcdefghi} (1.06)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	40.00 ^{nopqrstu} (0.68)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	44.00 ^{mnpqrst} (0.72)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	48.00 ^{klmnopqrs} (0.77)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
2	10	0.00 ^z (0.23)	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	20.00 ^{uvwxy} (0.46)	28.00 ^{stuvw} (0.55)	72.00 ^{cdefghij} (1.03)
	20	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	12.00 ^{wxyz} (0.37)	84.00 ^{abcde} (1.16)	88.00 ^{abcd} (1.20)	96.00 ^{ab} (1.30)
	30	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	32.00 ^{rstuvw} (0.58)	88.00 ^{abcd} (1.21)	88.00 ^{abcd} (1.20)	100.00 ^a (1.35)
	40	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	56.00 ^{ijklmno} (0.85)	88.00 ^{abcd} (1.21)	100.00 ^a (1.35)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	64.00 ^{efghijkl} (0.95)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	28.00 ^{stuvw} (0.54)	84.00 ^{abcde} (1.16)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)
3	10	0.00 ^z (0.23)	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	12.00 ^{wxyz} (0.37)	24.00 ^{tuvwx} (0.51)	76.00 ^{bcdefghi} (1.08)
	20	0.00 ^z (0.23)	0.00 ^z (0.23)	32.00 ^{pqrstuvw} (0.59)	56.00 ^{hijklmno} (0.86)	76.00 ^{cdefghi} (1.07)	80.00 ^{bcdef} (1.12)
	30	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	32.00 ^{rstuvw} (0.58)	68.00 ^{defghijk} (0.99)	88.00 ^{abcd} (1.20)	96.00 ^{ab} (1.30)
	40	0.00 ^z (0.23)	0.00 ^z (0.23)	56.00 ^{ijklmno} (0.85)	72.00 ^{cdefghij} (1.03)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	50	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	60.00 ^{ghijklmn} (0.89)	88.00 ^{abcd} (1.20)	100.00 ^a (1.35)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	20.00 ^{uvwxy} (0.46)	80.00 ^{bcdefg} (1.11)	100.00 ^a (1.35)	100.00 ^a (1.35)	100.00 ^a (1.35)

4	10	0.00 ^z (0.23)	0.00 ^z (0.23)	0.00 ^z (0.23)	4.00 ^{yz} (0.27)	24.00 ^{tuvwxy} (0.50)	20.00 ^{uvwxy} (0.46)
	20	0.00 ^z (0.23)	0.00 ^z (0.23)	24.00 ^{tuvwxy} (0.50)	12.00 ^{wxyz} (0.37)	80.00 ^{bcdefg} (1.11)	36.00 ^{opqrstuv} (0.64)
	30	0.00 ^z (0.23)	0.00 ^z (0.23)	40.00 ^{nopqrstu} (0.67)	48.00 ^{klmnopqrs} (0.77)	84.00 ^{abcde} (1.17)	84.00 ^{abcde} (1.16)
	40	0.00 ^z (0.23)	0.00 ^z (0.23)	52.00 ^{ijklmnopq} (0.81)	76.00 ^{bcdefghi} (1.07)	96.00 ^{ab} (1.30)	88.00 ^{abcd} (1.21)
	50	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	44.00 ^{mnpqrst} (0.72)	84.00 ^{abcde} (1.16)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	12.00 ^{wxyz} (0.37)	76.00 ^{cdefghi} (1.06)	92.00 ^{abc} (1.25)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)
5	10	0.00 ^z (0.23)	0.00 ^z (0.23)	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	12.00 ^{wxyz} (0.37)	16.00 ^{vwxyz} (0.42)
	20	0.00 ^z (0.23)	0.00 ^z (0.23)	0.00 ^z (0.23)	68.00 ^{defghijkl} (0.98)	48.00 ^{lmnopqrs} (0.76)	76.00 ^{bcdefgh} (1.08)
	30	0.00 ^z (0.23)	0.00 ^z (0.23)	20.00 ^{uvwxy} (0.46)	76.00 ^{bcdefghi} (1.07)	52.00 ^{ijklmnopqr} (0.81)	76.00 ^{bcdefghi} (1.07)
	40	0.00 ^z (0.23)	0.00 ^z (0.23)	32.00 ^{pqrstuvw} (0.59)	48.00 ^{klmnopqrs} (0.77)	92.00 ^{abc} (1.25)	80.00 ^{bcdef} (1.12)
	50	0.00 ^z (0.23)	0.00 ^z (0.23)	32.00 ^{qrstuvw} (0.58)	68.00 ^{defghijk} (0.99)	92.00 ^{abc} (1.25)	100.00 ^a (1.35)
	60	0.00 ^z (0.23)	8.00 ^{xyz} (0.32)	60.00 ^{ghijklm} (0.91)	76.00 ^{bcdefghi} (1.07)	96.00 ^{ab} (1.30)	100.00 ^a (1.35)

*Figures in the parentheses are arc sine transformed values

Values with the same letters are not significantly different at 5.00% level by LSD

Table 29. Effect of microwave radiation on moisture, gluten and vitamin B content of wheat flour

Treatment	Moisture content (%)	Dry gluten content (%)	Vitamin B (ppm)				
			B ₁	B ₂	B ₃	B ₅	B ₆
Control	10.63	7.95	11.90	0.03	14.17	—	4.28
T4	7.95	7.15	12.45	0.07	11.63	—	6.98
T5	9.43	7.84	12.70	0.04	12.42	—	7.24
't' statistics							
Control + T4	10.20*	1.62 ^{ns}	—	—	—	—	—
Control + T5	9.72*	0.31 ^{ns}	—	—	—	—	—

*Indicates significance at 5 % level (p<0.05)

^{ns} Indicates non significance at 5 % level (p>0.05)

Table 30. Effect of microwave radiation on colour of wheat flour

Treatment	Lightness (<i>L</i>)	Redness (<i>a</i>)	Yellowness (<i>b</i>)	Colour change (ΔE)
Control	85.69	1.95	13.20	—
T4	85.38	1.89	13.75	0.64
T5	85.58	1.94	13.59	0.41
't' statistics				
Control + T4	3.44 ^{ns}	6.00 ^{ns}	11.00 ^{ns}	7.44 ^{ns}
Control + T5	5.50 ^{ns}	1.70 ^{ns}	5.92 ^{ns}	8.30 ^{ns}

^{ns} Indicates non significance at 5 % level ($p>0.05$)



Plate 12. Normal grub and malformed grub



Plate 13. a



Plate 13. b

Plate 13. Normal and malformed pupa (a) dorsal view (b) ventral view



Plate 14. a



Plate 14. b

Plate 14. Normal and malformed adult (a) dorsal view (b) ventral view

Discussion

5. DISCUSSION

Microwave irradiation is the safe and modern curative method of disinfestation of stored grain pests. The dielectric heating generated during microwave irradiation kills the insect and affects the reproductive capacity of surviving insects (Vadivambal *et al.*, 2007; Patil *et al.*, 2020). Microwave irradiation helps in the management of both internal as well as external feeders (Patil *et al.*, 2020). The major advantage of microwave irradiation over the conventional management options such as insecticides and fumigants are

1. Lack of any residues after treatment
2. It does not affect human beings adversely
3. It does not affect the quality of the produce
4. Less time consuming,
5. Low energy consumption
6. Does not harm the environment
7. Low chances of development of resistance (Watters, 1976; Hurlock *et al.*, 1979; Vadivambal *et al.*, 2007; Patil *et al.*, 2020).

The results obtained in our study ‘Microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)’ is discussed in this chapter with the help of pertinent literature under the following headings.

- 5.1 Variation in the susceptibility of various stages of *Tribolium castaneum* to microwave radiation
- 5.2 Effect of power of microwave radiation on mortality of various stages of *Tribolium castaneum*
- 5.3 Effect of exposure time on mortality of various stages of *Tribolium castaneum*

5.4 Effect of thickness of flour bed thickness on mortality of various stages of *Tribolium castaneum*

5.5 Interaction of power, exposure time and flour bed thickness on mortality of various stages of *Tribolium castaneum*

5.6 Effect of microwave radiation on nutritional parameters of wheat flour

5.1 VARIATION IN THE SUSCEPTIBILITY OF VARIOUS STAGES OF *Tribolium castaeum* TO MICROWAVE RADIATION

The mortality of eggs, grubs, pupae and adults at 440 W power, three cm flour bed thickness and 30 s exposure was statistically analysed to assess the susceptibility of various developmental stages of *T. castaneum* to microwave irradiation (Fig.1). The pupal stage was the most tolerant to microwave irradiation, with mortality of 48.00 per cent. Adult mortality (68.00 per cent) was also comparable to pupal mortality. But mortality of egg (80.00 per cent) and grub (96.00 per cent) did not differ statistically from adult mortality.

The susceptibility of different life stages of insects to microwave radiation varies in different experiments and with different species of stored grain pests. Vadivambal *et al.* (2007) also reported that the larvae of *T. castaneum* are more susceptible over pupae and adults to microwave radiation and they argued that pupae are more tolerant to heat than other developmental stages. During microwave irradiation, the mortality of insects is due to the generation of dielectric heat (Vadivambal *et al.*, 2007). Microwave radiation causes the rotation of the most common dipole molecule, the water, resulting in heat generation (Mohapatra *et al.*, 2014). Therefore, the tolerance of the pupal stage to heating can be the possible reason by which the pupal stage is less susceptible to microwave irradiation. Patil *et al.* (2020) also reported that pupae of *T. castaneum* infesting almonds are least vulnerable to microwave irradiation. The LD₅₀ of microwave irradiation for 30 s with pupae of *T. castaneum* was 632.3 W, while it was 475.1 W for adults and 346 W for larvae. At 60 s exposure time pupae were significantly tolerant to microwave irradiation over larvae and adults. Vadivambal *et al.* (2010) also reported

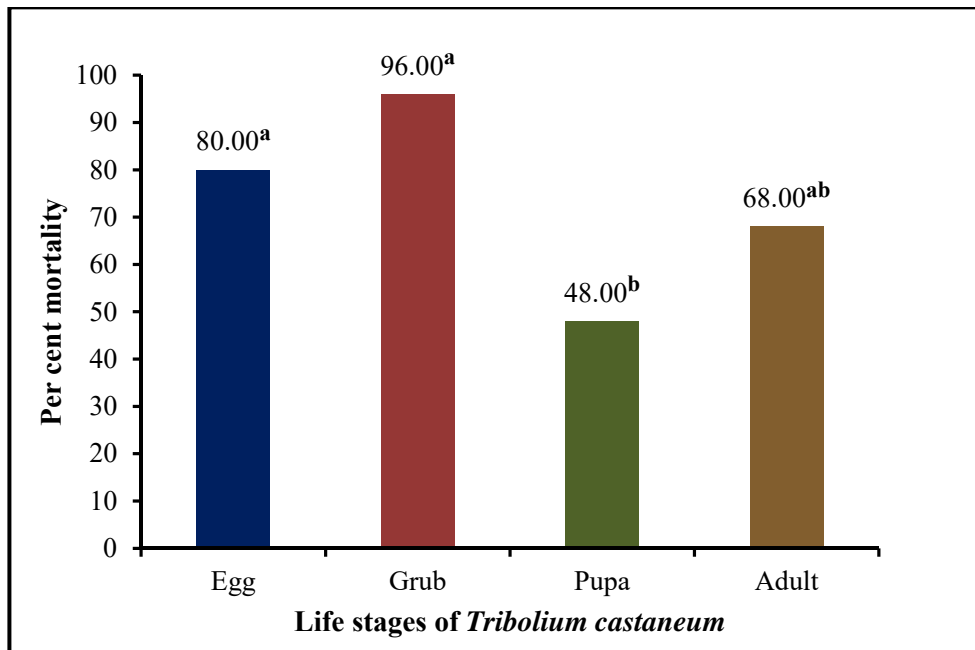


Fig 1. Susceptibility of various stages *Tribolium castaneum* to microwave radiation

that larval mortality of *T. castaneum* infesting corns was significantly higher than the adults. Manickavasagan *et al.* (2013) recorded that larvae of *T. castaneum* infesting dates were more susceptible than adults. Tayeb *et al.* (2018a) noted that adults are the most tolerant stage of *T. castaneum*. Similar results were also obtained by Meenatchi *et al.* (2015), while working with maize infested with *T. castaneum* larve, pupae and adults.

Among the various developmental stages of pulse beetle, *Callosobruchus maculatus*, eggs were the most susceptible stage, followed by larvae, pupae and adults. The larval and pupal mortality of *C. maculatus* did not differ significantly from each other (Purohit *et al.*, 2013). Shoughy and Elzun (2014) also reported eggs of *C. maculatus* as the most susceptible stage, followed by larvae, adults and pupae. Similar to pupae of *T. castaneum*, pupae of *C. maculatus* was also the least susceptible stage to microwave irradiation.

Tiwari *et al.* (2021) found that eggs of *C. maculatus* are the most susceptible stage and pupae are the least susceptible stage. The LT₅₀ at a power level of 230 W was 3.53 min for egg, 5.64 min for larva, 6.28 min for pupa and 5.35 min for adults. In the case of lepidopteran storage pest, fig moth, *Ephestia cautella* also, eggs were the most susceptible stage followed by female pupae, larvae and male pupae (Khalaf *et al.*, 2015).

Contrary to all these results, larvae and pupae of *Sitophilus oryzae* were more sensitive to microwave radiation than eggs (Franco *et al.*, 2013). The adults of *S. oryzae* were the most tolerant stage, according to their study.

5.2 EFFECT OF POWER OF MICROWAVE RADIATION ON MORTALITY OF VARIOUS STAGES OF *Tribolium castaneum*

In all the studies conducted with microwave radiation, insect mortality increase with the increase in power of microwave radiation (Vadivambal *et al.*, 2007; Vadivambal *et al.*, 2010; Singh *et al.*, 2012; Purohit *et al.*, 2013; Shoughy and Elzun, 2014; Khalaf *et al.*, 2015; Meenatchi *et al.*, 2015; Patil *et al.*, 2020), which is similar to our results. Vadivambal *et al.* (2010) obtained complete mortality of larvae and adults of *T. castaneum* at 600 W exposed for 14 s and at 500 W exposed for 28 s. The thickness of corn samples kept for irradiation was one cm. The current study also

recorded 100.00 per cent mortality of larvae and adults at a power level of 440 W with 20 s exposure and one cm flour bed thickness. At 616 W also, we recorded total mortality at the same exposure time and thickness.

Vadivambal *et al.* (2007) found complete mortality of larvae, pupae and adults of *T. castaneum* with 500 W power and at 28 s exposure. The mortality of larvae, pupae and adults in our study was 100.00 per cent at 440 W with 30 s exposure and one cm flour bed thickness, which is almost identical to the pupal mortality obtained by Vadivambal *et al.* (2007). To further support our findings, Meenatchi *et al.* (2015) obtained cent per cent mortality of larvae, pupae and adults of *T. castaneum* at 540 W and 20 s exposure.

Patil *et al.* (2020) recorded an exposure time of 90 s to cause 100.00 per cent mortality of *T. castaneum* adults at 360 W. We could record 96.00 per cent mortality of adults at 264 W with 50 s and one cm flour thickness. Hundred per cent mortality of larvae, pupae and adults occurred at 60 s exposure with 600 W power. An exposure time of 20 s was sufficient to cause 100.00 per cent mortality of larvae, pupae and adults at 616 W and one cm flour thickness. The study by Patil *et al.* (2020) does not mention the thickness of almonds kept for irradiation. The variation in the thickness may be the reason for the variation in results obtained between our study and Patil *et al.* (2020).

The eggs of *E. cautella* are more tolerant to microwave radiation compared to *T. castaneum*. With an exposure time of 10 s and 600 W power, the mortality of the eggs of *E. cautella* was 83.30 per cent (Khalaf *et al.*, 2015). But in our study, we obtained 96.00 per cent egg mortality of *T. castaneum* at 616 W, at 10 s exposure with one cm flour bed thickness.

Manickavasagan *et al.* (2013) irradiated a single layer of dates infested with adults of *T. castaneum* with microwave at four different power levels of 180, 300, 600 W and 800 W. At 180 W, mortality was 0.00 per cent even after exposing for 40 s. But in our study with 136 W power and 40 s exposure with one cm flour thickness, there was a mortality of 40.00 per cent adults. At the highest dose of 800 W, there was 100.00 per cent adult mortality at 40 s exposure, identical to our results. Similarly,

grubs also in both the studies recorded 100.00 per cent mortality with 30 s exposure and 800 W at a thickness of one cm.

5.3 EFFECT OF EXPOSURE TIME ON MORTALITY OF VARIOUS STAGES of *Tribolium castaneum*

All the previous studies unanimously agree on the increase in mortality of various stages of insects with an increase in exposure time, as we recorded in the current study. Vadivambal *et al.* (2007) irradiated wheat infested with larvae of *T. castaneum* at 400 W at 28 and 56 s, and the mortality increased from 91.00 per cent to 100.00 per cent. Our study recorded 100.00 per cent mortality at a power of 440 W at 30 s and 60 s exposure time at a flour bed thickness of one cm. We obtained 100.00 per cent pupal mortality at 440 W with an exposure of 30 s itself at a flour thickness of one cm. In contrast, Vadivambal *et al.* (2007) obtained only 76 per cent mortality of *T. castaneum* pupae at 400 W with 28 s exposure. But at 56 s, mortality was cent per cent, identical to our study. Irradiating adults of *T. castaneum* also recorded mortality of 86.00 per cent in the study of Vadivambal *et al.* (2007), at 400 W power with an exposure of 28 s, while we recorded 100.00 per cent mortality at 440 W with 30 s exposure and at one cm flour thickness. The slightly higher mortality obtained in our study may be due to the higher power (440 W) and exposure time (30 s).

Exposing adults of *T. castaneum* for 10 s at 616 W resulted in a mortality of 52.00 per cent at a flour bed thickness of one cm in our study. But Vadivambal *et al.* (2010) could record 100.00 per cent mortality of *T. castaneum* adults at a slightly lower dose of 600 W and an exposure time of 14 s. Increasing the exposure time to 28 s recorded cent per cent mortality of *T. castaneum* adults by Vadivambal *et al.* (2010). Our study also recorded 100.00 per cent mortality at 660 W, 30 s exposure one cm flour thickness.

LD₅₀ values of larvae, pupae and adults of *T. castaneum* infesting almonds decreased with increased exposure time (Patil *et al.*, 2020). An exposure of 60 s resulted in 100.00 per cent of larvae, pupae and adults at 480 and 600 W power of microwave radiation. Our study also recorded 100.00 per cent mortality of all developmental stages of *T. castaneum* at 440 W with an exposure of 60 s. With 30 s exposure,

Patil *et al.* (2020) could not achieve cent per cent mortality of any of the developmental stages of *T. castaneum* at 600 W. But we could record complete mortality of all the stages of *T. castaneum* at a slightly higher dose of 616 W, at 30 s with a flour thickness of one cm.

Meenatchi *et al.* (2015) recorded 80.00 per cent mortality of *T. castaneum* larvae at 270 W power with an exposure of 20 s. We could record higher larval mortality of 92.00 per cent at a power level of 264 W, 20 s and flour bed thickness of one cm. By increasing the exposure time to 30 s, Meenatchi *et al.* (2015) recorded 93.00 per cent mortality of *T. castaneum* larvae, whereas we could record 96.00 per cent at 264 W power, 30 s exposure and one cm flour thickness. Pupal mortality of *T. castaneum* in the studies of Meenatchi *et al.* (2015) with 20 s and 30 s exposure were 85.00 per cent and 95.00 per cent. In the current study, we observed 80.00 per cent and 88.00 per cent mortality of *T. castaneum* pupae with an exposure of 20 and 30 s, respectively, at a power of 264 W and flour bed thickness of one cm. The 20 s exposure resulted in 76.00 per cent mortality of *T. castaneum* adults in our study and the study of Meenatchi *et al.* (2015). The mortality increased to 96.00 per cent at 30 s exposure time in the study of Meenatchi *et al.* (2015), while the mortality did not increase from 76.00 per cent even with a 10 s increase in exposure time in our study. An exposure of 30 s with a power of 450 W was sufficient to cause complete mortality of larvae, pupae and adults of *T. castaneum* (Meenatchi *et al.*, 2015). Our study also recorded cent per cent mortality of all life stages at 440 W with 30 s exposure and one cm flour thickness.

An increase in exposure time resulted in greater mortality of all developmental stages in *Callosobruchus maculatus* also (Singh *et al.*, 2012; Purohit *et al.*, 2013; Tayeb *et al.*, 2018b). Similarly in *E. cautella* there was increased mortality of eggs larvae and pupae with increase in exposure time and poer levels of 600, 800 and 1000 W (Khalaf *et al.*, 2015).

5.4 EFFECT OF FLOUR BED THICKNESS ON MORTALITY OF VARIOUS STAGES OF *Tribolium castaneum*

Most of the studies used a thin layer of grains for microwave irradiation. The influence of the increase in grain bed thickness was studied by Mohapatra *et al.* (2014) in green gram infested with *C. maculatus* and recorded a decrease in mortality of *C. maculatus* with an increase in grain bed thickness. The heat generation by microwave depends on the produce load, moisture content and composition of the biological material. An increase in the load reduces heat generation, decreasing the mortality of insects. Higher microwave energy is required with an increase in the load to generate heat sufficient for insect mortality (Mohapatra *et al.*, 2014).

5.5 EFFECT OF INTERACTION OF POWER, EXPOSURE TIME AND FLOUR BED THICKNESS ON MORTALITY OF VARIOUS STAGES OF *Tribolium castaneum*

The two factor interactions as well as three factor interactions were significant in causing the mortality of eggs of *T. castaneum*. The interaction of power \times thickness (df = 20, F = 8.95, P < 0.05), power \times exposure time (df = 25, F = 23.66, P < 0.05), thickness \times exposure time (df = 20, F = 3.27, P < 0.05) and power \times thickness \times exposure time (df = 100, F = 4.23, P < 0.05) was significant at 0.001 level.

Unlike egg, pupa and adult, interaction of flour bed thickness \times exposure time was non-significant with larval mortality (df = 20, F = 1.50, P = 0.07), while the interaction of power \times flour bed thickness (df = 20, F = 20.27, P < 0.05), power and exposure time (df = 25, F = 27.51, P < 0.05) and power \times flour bed thickness \times exposure time (df = 100, F = 4.00, P < 0.05) was significant at 0.001 level.

The interaction effect in causing pupal mortality was also significant at two factor and three factor levels *ie.*, power \times flour bed thickness (df = 20, F = 8.35, P < 0.05), power and exposure time (df = 25, F = 17.64, P < 0.05), flour bed thickness \times exposure time (df = 20, F = 3.23, P < 0.05) and power \times flour bed thickness \times exposure time (df = 100, F = 4.00, P < 0.05) were also significant at 0.001 level.

The interaction of power \times flour thickness (df = 20, F = 6.56, P < 0.05), power \times exposure time (df = 25, F = 14.14, P < 0.05), flour thickness \times exposure time (df = 20, F = 1.74, P < 0.05) as well as the three factor interaction (df = 100, F = 1.93, P < 0.05) were significant in causing mortality of adults of *T. castaneum*.

Valizadegan *et al.* (2011) noted a significant interaction between power and exposure time in causing adult mortality of saw toothed beetle, *Oryzaephilus surinamensis* and cigarette beetle, *Lasioderma serricorne*.

Singh *et al.* (2012) recorded that adults of *C. maculatus* move towards the surface of pulses during microwave radiation, resulting in higher mortality. During our experiments, the grubs remain at the bottom of the beaker during irradiation, reducing the effect of irradiation even with an increase in exposure time. The immobile stages, such as eggs and pupae, were evenly distributed in the flour, while the adults moved towards the surface layers of flour during irradiation. Thus, the non-significant interaction between the two factors, flour bed thickness and exposure time, in causing larval mortality may be due to the congregation of grubs at the flour bottom.

Microwave irradiation reduces the moisture content and body pH of adults of *Tribolium castaneum* (Lu *et al.*, 2010). Lu *et al.* (2010) found that peroxide content in irradiated adults of *T. castaneum* increases with an increase in irradiation dose, while the alkaline phosphatase activity decreases with the increase in microwave radiation power. Aggregation and degradation of DNA was also reported in microwave irradiated adults of *T. castaneum* (Lu *et al.* 2010; Tayeb *et al.* 2018a). Microwave irradiation changed the amino acid composition in irradiated *T. castaneum* adults. Though microwave irradiation leads to dielectric heating in insects, peroxide level, pH, alkaline phosphatase and acetylcholine esterase activity varied from adults subjected to conductive heating at a constant temperature of 65°C. Hence, dielectric heating by microwave radiation differs from normal conductive heating. The microwave irradiation can cause the mortality of *T. castaneum* at a temperature lower than that caused by conductive heat treatment (Lu *et al.*, 2010).

Lu *et al.* (2011) reported changes in elytra and at the cellular level of *T. castaneum* upon microwave heating. Inside the nucleus, water evaporation led to the

formation of empty vacuoles and the destruction of mitochondria and Golgi bodies. Microwave irradiation of *T. castaneum* adults increased the esterase enzyme activity (Tayeb *et al.*, 2018a). The heating of insects due to microwave radiation leads to an increase in the expression of heat shock protein (Tungjitwitayakul *et al.*, 2016).

Pandir and Guven (2014) studied the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) in the irradiated larvae of *E. kuehniella*. Microwave irradiation resulted in a decrease in SOD, CAT and GPx, but increased MDA level. They observed damage to DNA after exposure of larvae to 600 W for 50 s. The lower level of microwave irradiation did not cause any significant damage to DNA. Hence we can conclude that mortality of various stages of *T. castaneum* may be due to the structural, cellular and enzymatic changes induced by microwave radiation.

5.6 EFFECT OF MICROWAVE RADIATION ON NUTRITIONAL PARAMETERS OF WHEAT FLOUR

5.6.1 Effect of microwave radiation on the moisture content of wheat flour

Microwave irradiation at 800 W for 50 s at four cm and five cm flour bed thickness significantly decreased the moisture content of wheat flour. The decrease in moisture content can be due to the dielectric heating of flour caused by microwave radiation. Inthujaa *et al.* (2019) also recorded a significant reduction in moisture content after irradiating wheat flour at 700 W for 60 s.

Purohit *et al.* (2013) reported 0.40 per cent moisture loss from mung bean (*Vigna radiata*) irradiated at 400 W for 28 s. Similarly, Meenatchi *et al.* (2015) also recorded a loss in moisture from maize due to microwave irradiation. Moisture loss at 360 and 450 W power was 1.00 per cent, whereas, at 540 W, it was 4.00 per cent.

5.6.2 Effect of microwave radiation on gluten content of wheat flour

Though there was a slight decrease in gluten content due to microwave irradiation, the decrease was not statistically significant. Qu *et al.* (2017) recorded a significant decrease in the gluten content of wheat kernels when irradiated at 700 W for

greater than or equal to 30 s. Tao *et al.* (2021) also recorded a significant decrease in gluten content due to microwave irradiation. Qu *et al.* (2017) irradiated wheat kernels with a moisture content of 18-20 per cent. Higher moisture content leads to a higher level of temperature in the irradiated material because of dielectric heating destroying the gluten in wheat. Tao *et al.* (2021) reported the moisture content of wheat bran to be the most important factor that influencing the gluten quality of microwave irradiated wheat bran.

Kaasova *et al.* (2002) irradiated sprouted wheat with 10.00 per cent moisture content and 15.00 per cent. At 10.00 per cent moisture content, the temperature was 60°C, and at 15.00 per cent moisture content, the temperature was 80°C. There was a greater decrease in gluten content when the moisture content was 15.00 per cent, and the temperature was 80°C when compared to the moisture content of 10.00 per cent and a temperature of 60°C.

The moisture content of wheat flour used in our study was 10.63 per cent. The lower level of moisture in the wheat flour may not have raised the temperature sufficient enough to affect the gluten content of wheat flour.

5.6.3 Effect of microwave radiation on the colour of wheat flour

In the current study, the lightness (*L*) and redness (*a*) of wheat flour slightly decreased in a non-significant manner upon microwave irradiation of wheat flour at 800 W for 50 s at four cm and five cm flour bed thickness, while the yellowness (*b*) recorded a non-significant increase after microwave irradiation. The colour difference (ΔE) was also non-significant over control.

Warchalewski *et al.* (1998) reported that colour difference (ΔE) in microwave irradiated wheat grain was significant only when the duration of irradiation was greater than 120 s. Similar to our results, Velu *et al.* (2006) reported a decrease in the value of *L* and an increase in the value of *b* in flour obtained from microwave dried maize.

5.6.4 Effect of microwave radiation on vitamin B of wheat flour

The level of thiamine (vitamin B₁), riboflavin (vitamin B₂) and pyridoxine (vitamin B₆) slightly increased upon microwave irradiation. But, there was a reduction in the content of niacin (vitamin B₃) after irradiation.

Several reports stated that microwave cooking of food products did not significantly affect vitamin B content. There was better retention of vitamin B complex in microwave heating than the conventional heating method (Cross and Fung, 1982; Brown *et al.*, 2020). Also, Graham (1977) reported that thiamine content (vitamin B₁) in convenient frozen foods remained unaffected even after microwave cooking, and in some instances, a slight increase in thiamine content was recorded, which is similar to our study. Cross and Fung (1982) found that pyridoxine (vitamin B₆) content is also unaffected by microwave heating. Cross and Fung (1982) also reviewed the effect of microwave irradiation on vitamin content and reported that thiamine content could increase or decrease after irradiation. In our study, there was a slight increase in vitamin B₁, B₂ and B₆ in irradiated treatments compared to control. This increase in vitamin B₁, B₂ and B₆ might be due to the reduction in moisture content of wheat flour during irradiation. The decrease in moisture content must have increased the net vitamin B₁, B₂ and B₆ content. Bowers *et al.* (1974) also reported that pyridoxine content could differ due to the variation in moisture content. A few other studies also reported a higher value of vitamin B₆ after microwave irradiation (Bowers *et al.*, 1974; Wing and Alexander, 1972)

Cross and Fung (1982) reported some loss in vitamin B₃ as they are water-soluble. But, Baldwin *et al.* (1976) reported that B₃ increased in microwave cooked meat samples compared to raw meat. In our study, we recorded a slight reduction in vitamin B₃ due to irradiation. Vitamin B content could vary according to the commodity difference, shape and size, and variation in cooking time and temperature during treatment (GovHK, 2005) and reported equal or better retention of vitamins in microwave cooking.

Summary

6. SUMMARY

Wheat holds a unique position in the daily diet and has a significant role in ensuring food security and nutritional security. But the crop faces severe loss during the post-harvest phase mainly due to insects. Red flour beetle, *Tribolium castaneum*, is a cosmopolitan stored grain pest causing severe economic loss to cereal-based products, especially wheat flour. Chemical fumigation, the extensively used approach to manage storage pests, led to resistance development in insects, residual effects on products, and adversely affected human health and the environment. Hence, there is a constant demand for novel, eco-friendly, and economic strategies for storage pest management. Thus, the current study assessed the efficacy of microwave radiation for the management of storage pests. The findings thus obtained are summarized below.

All the developmental stages of *T. castaneum* were irradiated at five different microwave powers (136, 264, 440, 616 and 800 W) by varying the flour bed thickness of wheat flour at five levels (1, 2, 3, 4 and 5 cm) and exposure time at six levels (10, 20, 30, 40, 50 and 60 s) along with unirradiated control.

Eggs (two days old), grubs (20±2 day old), pupae and adults (17±2 day old) of *T. castaneum* were irradiated in a household microwave to assess the susceptibility of various life stages. Larvae were most vulnerable to irradiation. Susceptibility of eggs and adults were comparable to each other. But, the pupal stage exhibited relatively higher tolerance to microwave treatment.

Microwave power was directly proportional to the mortality of all life stages of *T. castaneum*. The mortality of egg, pupa and adult was highest at 800 W power level, whereas pupal mortality was comparable and higher at 616 and 800 W. The death rate of all developmental stages was highest at the lowest flour bed thickness of one cm. Similar to the effect of microwave power, mortality increased significantly with an increase in exposure time from 10 to 60 s. At the longest exposure time (60 s), egg, grub and adult mortality was high and pupal mortality at 50 and 60 s was comparable.

The analysis on the interaction of power and thickness revealed that mortality of all stages of *T. castaneum* increased with an increase in power level and decrease in

flour thickness. In power-time interaction, higher irradiation dose and extended exposure period resulted in higher mortality of *T. castaneum*. The interaction effect of thickness and time showed that mortality was higher at lower flour bed thickness and longer exposure time. The analysis on the interaction of power, thickness and time recorded higher mortality at higher dose, lower thickness and longer exposure period of microwave radiation.

Irradiating wheat flour in thin layers (one, two and three cm) requires more cycles to complete irradiation which will be a time and power-consuming process. By considering this practical difficulty, microwave treatment at two higher flour bed thickness of four and five cm at 800 W for 50 s were selected as the best. Both the treatments were analysed for colour, moisture, gluten and vitamin B content and compared with unirradiated control. There was a significant reduction in moisture content, whereas gluten content and colour was unaffected by irradiation. In addition, there was slight increase in vitamin B₁, B₂ and B₆ and a slight decrease in vitamin B₃ in treated samples compared with control.

There was better enrichment of vitamins and relatively low moisture loss in treatment at five cm flour bed thickness than treatment at four cm flour thickness. Hence, microwave irradiation of wheat flour at 800 W for 50 s at a flour thickness of five cm can be recommended as the best treatment combination for the management of red flour beetle because of the efficacy, ease of application, lack of residual effect without compromising the nutritional quality of wheat flour.

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**Microwave radiation for the management of red
flour beetle, *Tribolium castaneum* (Herbst)
(Coleoptera: Tenebrionidae)**

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ABSTRACT OF THE THESIS

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ABSTRACT

Red flour beetle, *Tribolium castaneum*, is a cosmopolitan stored grain pest imparting severe economic loss in cereal-based products, especially wheat flour. Microwave irradiation, an eco-friendly physical management strategy without any residual effect on food and less resistance development, can be an alternative to chemical control measures. Hence, the study entitled “Microwave radiation for the management of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)” was conducted to assess the effectiveness of household microwave system for the management of red flour beetle, *T. castaneum* and to study the effect of microwave radiation on nutritional factors of wheat flour.

The experiment was carried out from January 2021 to October 2021 at Pesticide Residue Testing Laboratory, Department of Agricultural Entomology, and at the Agri-Business Incubator, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. All the developmental stages of *T. castaneum* were irradiated at five different microwave powers (136, 264, 440, 616 and 800 W) by varying the flour bed thickness of wheat flour at five levels (1, 2, 3, 4 and 5 cm) and exposure time at six levels (10, 20, 30, 40, 50 and 60 s) along with unirradiated control.

Eggs (two days old), grubs (20±2 day old), pupae and adults (17±2 day old) of *T. castaneum* were irradiated in a household microwave to assess the susceptibility of various life stages. Larvae were most vulnerable to irradiation. Susceptibility of eggs and adults but were comparable to each other, while the pupal stage exhibited relatively higher tolerance to microwave treatment.

Microwave power was directly proportional to the mortality of all life stages of *T. castaneum*. The mortality of egg, pupa and adult was highest at 800 W power level, whereas pupal mortality was comparable and higher at 616 and 800 W. The death rate of all the developmental stages was highest at the lowest flour bed thickness of one cm. Similar to the effect of microwave power, exposure period and mortality was

directly proportional to each other. At the longest exposure time (60 s), egg, grub and adult mortality was high and pupal mortality at 50, and 60 s was comparable.

The analysis on the interaction of power and thickness revealed that mortality of all stages of *T. castaneum* increased with an increase in power level and decrease in flour bed thickness. In power-time interaction, higher irradiation dose and longer exposure period resulted in higher mortality of *T. castaneum*. The interaction effect of thickness and time showed that mortality was higher at lower flour bed thickness and longer exposure time. The analysis on the interaction of power, thickness and time recorded higher mortality at higher dose, lower thickness and longer exposure period of microwave radiation.

The best two treatment combinations that recorded complete mortality of all life stages of *T. castaneum* was 800 W irradiation dose exposed for 50 s at higher flour bed thicknesses of four and five cm. The comparison of nutritional parameters of the two treatments with control indicated a decrease in moisture content. The gluten content and colour were unaffected by irradiation. There was an increase in vitamin B₁, B₂ and B₆ and a slight decrease in vitamin B₃ content in treated samples compared with control.

Microwave irradiation of wheat flour at 800 W for 50 s at a flour thickness of five cm is the best treatment combination for the management of red flour beetle because of the efficacy, ease of application, lack of residual effect without affecting the nutritional quality.