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EFFECTIVENESS OF A MICROWAVE FLUIDISED BED DRYER IN ERADICATION OF SEED-BORNE BOTRYTIS GREY MOLD OF LENTIL

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Abstract
A single mode microwave cavity, with a 2.45 GHz microwave source, was modified to have a microwave fluidized bed and its potential to eliminate Botrytis grey mold (BGM) pathogen of lentil seeds was evaluated. Air speed was maintained at a constant value and was just enough to fluidize 100g of red lentil seeds in the sample holder. Two wet-based (w.b.) seed moisture contents (MC) of 10.5% and 18.5% were prepared and the process parameters were selected as air temperature at 50 and 60°C; microwave power at 0, 300, 400 W for 18.5% MC and 0, 400, 500 W for 10.5% MC; and exposure times of 5 and 10 min. The effect of the process parameters on seed moisture loss, seed germination, the electrical conductivity of seed soaking water and the percentage of infected seeds (IS%) were analyzed. The most effective factors on moisture loss, after seed moisture content, was exposure time, followed by microwave power and air temperature. While the final bed temperature was mostly affected by air temperature, and then by microwave power. Furthermore, based on general full factorial regression and Pareto chart of standardized effects, moisture content had a major influence on the reduction of IS%. Seed pathogen inoculum reduction, without significant seed viability loss, was obtained by applying microwave power of 300W and set air temperature of 60°C (actual inlet air temperature of 57±1°C) on seeds with MC of 18.5% for 10 min. This gave a 27% reduction in IS%, from 82% to 55%. Thus, these results showed that applying microwave fluidized bed dryer was able to reduce BGM pathogen in lentil seeds and it could be considered as part of integrated disease management.

Introduction

Botrytis cinerea (BC) is one of the important seed-borne pathogens causing botrytis grey mold (BGM) disease of chickpea and lentil crops, one of the economically important seed-borne diseases in Australia. It can survive in the form of micro and macroconidia, mycelium, sclerotia and chlamydospores (Yigal Elad 2007). Lindbeck, Bretag et al. (2009) showed that BC is mostly located externally on lentil and chickpea seeds. They mentioned that seeds, which are infected during early stages of growth, may have an internal infection and will be discoloured and shrivelled, so they could be recognized by their appearance. However, those infected later, close to the harvest time, do not have any symptoms and might be used for sowing in the next season. The later seeds are mostly externally infected.

BGM control in lentil crop consists of integrated management including the application of resistant cultivars, healthy seeds, biological agents, seed treatment, foliar treatment with fungicides and cultural practices (Beniwal and Trapero-Casas 1994, Yigal Elad 2007). Lentil varieties grown in Australia do not have complete resistance against BGM and need fungicide application to control the disease (Lindbeck, Bretag et al. 2008). Therefore, using chemicals for seed and foliar treatments has remained the easiest and the most popular way of BGM control in this crop. However, there are still concerns about BGM pathogen's ability to develop resistance as well as environmental and health impacts due to over usage of

fungicides (Fillinger and Elad 2016). There have been some attempts to find a physical treatment for control or eradication of BGM pathogen. Burgess (1997) showed that BC is mostly present externally in chickpea seeds and can be controlled by moist heat at 45°C for 10 min or 50°C for 5 min. These treatments decreased seed infection to 2.5 and 0% respectively with no adverse effect on the seed germination and seedling emergence. However, the same treatment on the freshly harvested seeds reduced the infection to 18%, which could be the result of initial inoculum or changes in heat tolerance of the pathogen during treatment time. They also indicated that infected seeds did not necessarily have any visual symptoms to distinguish them from healthy seeds. This highlighted the need for seed testing. Marquenie, Lammertyn et al. (2002) applied UV-C, thermal treatment and their combination to inactivate spores of BC. They used first-order kinetic models to describe the inactivation of the spore, with temperature and UV dose as the independent factors for thermal and UV-C treatment respectively. They observed that there was no survival of conidia at 45°C after 15 min and at 48°C after 5 min. It was concluded that the combination of UV and thermal treatment allowed inactivation at lower temperature and lower UV doses. Nevertheless, there is still a lack of information on a proper physical method for elimination of BC from important legume crop seeds, such as lentil.
The objective of this experiment was to investigate the possibility of control or eradication of seed-borne *BC* from lentil seeds in a microwave fluidized-bed drier. For this purpose, the effect of different process parameters on moisture loss, temperature, seed viability and infected seed percentage (IS%) were investigated.

Materials and Methods

Lentil Seeds preparation

Red lentil seeds (Type Bolt) were purchased from PBseeds Co, Horsham, Victoria, Australia. The initial moisture content was measured by drying 10 g of unground seeds at 130℃ for 20h (Tang and Sokhansanj 1991). The 100-seeds weight was 4.27±0.09 g and their average diameter and thickness, which were measured using a digital calliper, were 5± 0.19 and 2.4 ± 0.14 mm (average of twenty seeds). To reach the desired moisture content, a calculated volume of sterilised distilled water was added to a known amount of dried seeds in a resealable polyethylene bag and after thorough shaking, they were kept in a refrigerator for at least one week and used in the experiment within one month, to make sure the seed viability did not change in storage, especially for moisture contents of above 17%. During the first week of storage, before the start of the experiment, seeds were mixed two times per day to make sure moisture was uniformly distributed among the seeds.

Inoculation of lentil seeds

Botrytis cinerea (174/02) spores, isolated from lentil seeds in Australia in 2002, were provided by Dr. Jenny Davidson, from the South Australian Research and Development Institute (SARDI). The isolate was subcultured on potato dextrose agar (PDA) and incubated at 22°C, 12h/12h dark/light cycle under fluorescent (OSRAM TLD/18W) and near ultraviolet (UV) lights (PHILIPS BLB/18W). After two weeks, when the cultures were full of conidia, the spore solution was prepared by flooding with sterilized distilled water (SDW) (with 0. 01% tween 20) on plates of BC on PDA and harvesting the spores by gently rubbing the surface of the culture using a glass rod. About 100 ml of spore solution, with 10^5-10^6 spore/ml, was obtained from two plates.
Inoculation of the lentil seeds was done according to the method described by Burgess, Bretag et al. (1997). Briefly, lentil seeds were surface sterilized with Sodium Hypochlorite (1%) for one minute, followed by rinsing three times with sterile distilled water. Sterilized

seeds were placed on a sterilized cheesecloth in a safety biological cabinet to let the excess water dry before putting them inside an autoclaved Scott bottle. The spore solution was diluted with sterilized distilled water to reach a concentration of 10^4-10^5 spore/ml. This solution was poured onto the lentil seeds in the Scott bottle to reach 10^3 spores/seed. The spore solution's volume was enough to wet all the seeds (for red lentil 25ml/100g). After pouring the spore solution, the bottle was tightly closed and mixed thoroughly and left overnight in the dark to make sure the spores are attached to the seeds. The bottle was shaken two times to make sure the spores are attached to all the seeds. On the next day, the inoculated seeds were spread on sterilized aluminum foil in a biological safety cabinet and weighed regularly to reach the required moisture content of 10.5 and 18.5% (wb). To reach the initial moisture content, they were left for three nights. After reaching the desired moisture contents, seeds were transferred in the Scott bottles, kept at 5°C and used in the experiment within one month. Before each treatment, seeds were taken out of the refrigerator and left overnight in the room to reach room temperature.

Microwave fluidized bed system and treatment of the lentil seeds

Schematic of the system is illustrated in Figure 1.

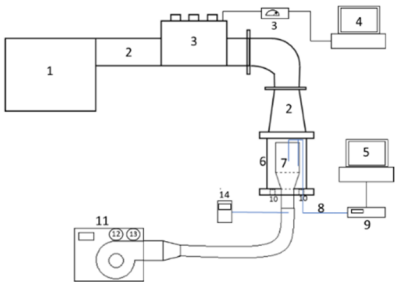

Figure 1 Microwave fluidized-bed system for drying and disinfection of particles, 1- magnetron, 2- waveguide, 3- three-stub tuner, 4- absorbed power monitoring, 5- data acquisition system and microwave power control, 6- microwave cavity, 7- sample holder (fluidized bed), 8- fibre optic probes, 9- temperature monitor, 10- vents, 11- air blower and heater, 12- air speed potentiometer, 13- air temperature potentiometer, 14- inlet air temperature monitor

For each treatment, 90g of healthy seeds were mixed with 10g of inoculated seeds, which were marked with a red marker. The total of 100g of seeds was transferred into the sample holder with a perforated bottom. Three fiber optic sensors were placed in the hot spot (top center of the bed), the cold spot (corner of the cylindrical sample holder) and at the bottom of the sample holder, where the air enters. This was considered as inlet air temperature. Hot and cold spots were obtained from the results of simulation of microwave power distribution in XFdtd® 7.5.0 (Remcom, Inc., State College, PA) using dielectric properties (Taheri, Brodie et al. 2018) and physical properties (Tang, Sokhansanj et al. 1991, Gharibzahedi, Ghahderijani et al. 2014) of red lentil seeds. Autodesk Inventor Professional 2017 (Autodesk Inc., USA) was used to design the 3-D geometry of lentil seeds with dimensions of 6.8 and 2.4 mm and 36 mm bed thickness (15 layers of lentil seeds), which was then placed in the sample holder inside the microwave cavity to perform the simulation.
Here, the real dimensions of the lentil seeds were not considered, because the real dimensions were too small, and the computer's space was not enough to solve the finite-

difference-time-domain equations. However, the thickness of 36mm was the same as 100g of red lentils at 18.5% MC, which was used in the experiment. The amount of sample was selected, firstly, for having a proper power density, which did not increase the hot spot temperature quickly and harm the seeds and secondly, for having a thickness less than the penetration depth of the microwave at 2450 MHz in lentil seeds (Taheri, Brodie et al. 2018). As the seeds are fluidized by hot air, fiber optic probs did not show the real seed surface temperatures and it is assumed that the measured temperatures are an average of air and seeds surface temperature, which is henceforth called bed temperature. Air speed was adjusted to fluidize the bed. Turning the potentiometer, a fracture below 560 l/min turned the fluid bed into a static bed.

Evaluation of lentil seed viability and vigour after treatments

Seed viability was examined by the standard germination test. Twenty seeds per replicate and a total of 60 seeds per treatments were placed on Whatman filter paper no.1 in 9-cm Petri dishes. Each plate contained 10 seeds which were wetted by 4 ml of sterilized distilled water. Germination of the seeds was examined after two and five days. Vigour of the seeds was evaluated by measuring electrical conductivity (EC) of the seed soaking water according to ISTA (2016). Following each treatment, 50 lentil seeds were counted and, accurately weighed and placed in 250-ml Erlenmeyer flasks. One hundred ml distilled water was poured in each container to cover the seeds and provide enough depth to soak the seeds. The containers' heads were covered with aluminium foil and after 24 h ±, 15 minutes electrical conductivity of the soaking water was measured using an EC probe (model HI98304, HANNA Instruments Inc.; Melbourne, Australia) with a resolution of 0.01 mS/cm and accuracy of ±2% F.S. at 25°C. The result was calculated according to equation (1). Distilled water was used as a blank for background reading of conductivity and the test was carried out in triplicate.

$$Conductivity\ (\mu S\ cm^{-1}\ g^{-1}) = \frac{conductivity\ reading\ \left(\frac{\mu S}{cm}\right) - \ background\ reading}{weight\ of\ seeds} \quad (1)$$

Evaluation of BGM pathogen viability after treatments

Botrytis selective media (BSM) was prepared according to Burgess (1997) and Edwards and Seddon (2001). The media contained (g/l): glucose 2; PDA 5; Agar 10; tannic acid 5; Ridomil 0.02; Pentachloronitrobenzene (PCNB) solution 0.5% in ethanol 4; Zineb 0.00091; and streptomycin sulphate 0.1. a mixture of glucose. The PDA and Agar were autoclaved before adding to the rest of the ingredients.

After each treatment, the contaminated seeds, which had been marked, were separated and 30 seeds per replicate with a total of 90 seeds per treatment were subcultured on BSM. The plates were incubated at 23℃ and after 4 and 10 days were examined to see the pathogen's growth. BC was recognized, based on the appearance of the mycelium and their conidia under microscope (after 2 weeks) as well as the colour change of the selective media to brown, which was the result of tannic acid oxidation.

Evaluation of thermal resistance of the BMC pathogen in a water bath

To see the susceptibility of the pathogen to thermal treatment at different seed moisture contents, five grams of inoculated lentil seeds at moisture contents of 10.5% (initial), 12.5%, 14.5%, 16.5% and 18.5% (wb) were placed inside a resealable polyethylene bag and immersed in a water bath at temperatures 60, 65, 67, 70, 75 ℃ for 10 min. The seeds were

sub-cultured on BSM and examined for the percentage of infected seeds after 4 and 10 days. Five-Minute exposure time was also examined for those temperatures, which were effective in 100% elimination of the pathogens. The results of these treatments helped the selection of the microwave power to treat the seeds with selected moisture content. The powers were selected to raise the seed surface temperatures to those which were lethal to the pathogen so that the bed temperature was at least 5℃ above these temperatures.

Experimental design and statistical analysis

To investigate the effect of different process parameters on moisture loss, final temperature, germination, and frequency of pathogen isolation from the seeds or percentage of infected seeds (IS%), an experiment was designed with four factors, each with 2 or 3 levels and with 3 replications. Factors and levels were selected as follow: moisture content (MC) at 10% (dry form) and 18% wet base (wb); air temperature set at 50 and 60 ℃; microwave output power at 0, 200, 300 for 18% MC and 0, 400, 500 for 10% MC; and exposure times of 5 and 10 min. The set air temperature of 50 and 60℃ was measured as 50±1 and 57±1℃ at the inlet of the sample holder.

This experiment was set out as a randomized full factorial design in Minitab version 18.1 (Minitab Inc., Pennsylvania, USA). The effect of microwave power, air temperature, exposure time and initial moisture content on final sample temperature, moisture loss, seed viability and percentage of infected seeds were investigated using a general factorial regression and analysis of variance. The most effective factors were found from a Pareto chart of the standardized effects with $\alpha = 0.05$. Significant differences from the control were analyzed by one-way analysis of variance. All the statistical analysis was performed in MINITAB Statistical Software version 18.1 (Minitab, Inc., Pennsylvania, USA) and presented data are mean of three replicates.

Results and discussion

Simulation of power density inside the single mode cavity

Results of microwave power dissipation inside 100g of lentil seeds are shown in Figure 2. As can be seen in the power distribution in the bottom layer of the lentil seeds (Figure 2 (a)), the hot spot of the microwave radiation is predicted to be in the centre of the sample and the cold spot is along the circumference of the cylindrical sample holder. The same distribution was obtained for the top layer of the samples (data not shown). In Figure 2 (b), the maximum and average predicted microwave power density are represented. Here, the maximum microwave power is predicted to be on the top layer of lentil samples and the minimum power is absorbed in the middle layers, 20-25 mm distance from the top. Therefore, the hot spot was in the centre of the top layer and the cold spot in the corner of the middle layer. Fibre optic probes were placed in these two locations to obtain hot and cold spot temperatures during the treatments. Additionally, hot air was assumed to be evenly distributed in the bed after entering through the perforated plate.

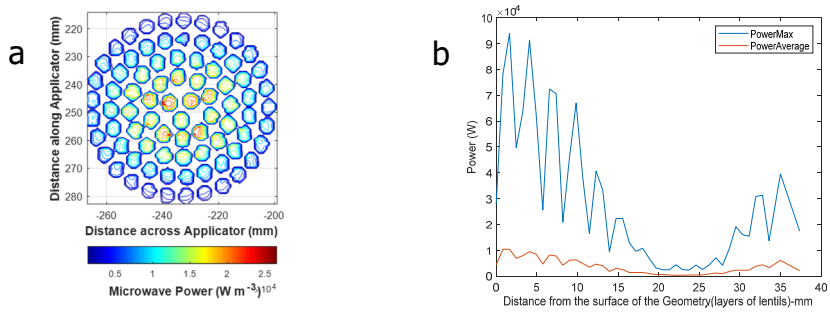

Figure 2 Power dissipation in lentil seeds, the result of simulation; (a) absorbed power distribution in a cross section of the bottom layer of lentil seeds; (b) vertical maximum and average power density in 100g of lentil seeds in a cylindrical sample holder (W/m^3)

Thermal sensitivity of the BGM pathogen in a water bath

Results of thermal sensitivity of the BGM pathogen on lentil seeds, with different seed MCs, are shown in Figure 3. A 10 min exposure time led to complete eradication of the pathogens from the lentil seeds at 65℃ for seed MC of 18.5%, 16.5%, and 14.5%, at 70℃ for 12.5% seed MC and at 75℃ for 10.5% MC (Figure 3(a)). A temperature of 65℃ was also lethal to seed pathogen after 5 min exposure of the contaminated seeds at 18.5% and 16.5% MC. However, seeds with MC of 14.5%, 12.5%, and 10.5% required temperatures of 70℃, 75℃ and 80℃ , respectively to remove the pathogen completely, after 5min. Taheri, Brodie et al. (2019) also found that Ascochyta blight pathogen of lentil seeds could be reduced more at higher seed moisture content and the same final temperature, by applying microwave treatment.

It must be noted that, here, the seeds were not directly exposed to water, which creates 100% water activity and lowers the lethal temperature to its minimum. The seeds were sealed in the resealable polyethylene bag and the moisture was not allowed to scape, and water activities could be considered as the seeds water activity at the corresponding moisture content at each temperature. If the exposure time is enough to reach the equilibrium in the closed bags, relative humidity (RH) might be considered close to the water activity.

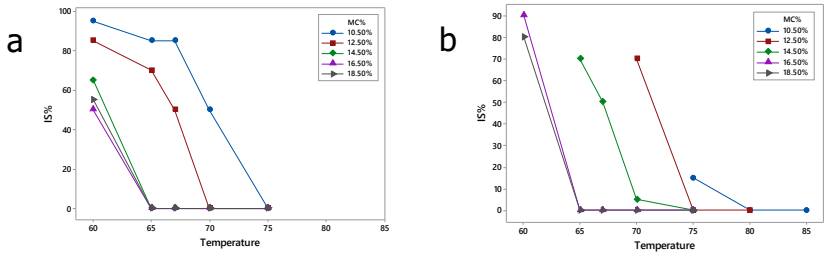

Figure 3 Percentage of botrytis grey mold infected lentil seeds after treatments in a water bath at different temperatures (°C) after (a) 10 min and (b) 5 min. IS%- infected seeds percentage; MC%-seed moisture content(w.b.)

Effect of microwave power, air temperature and exposure time on moisture loss and final temperatures of lentil seeds

Results of the Pareto chart of the standardized effects from general factorial regressions ($\alpha = 0.05$) for moisture loss and bed temperature are represented in Figure 4, (a) and (b) respectively. The x-axis of these charts shows the coefficient of each factor in the regression model divided by its standard error. The factors, with the standardized effect of more than 2.04 (red vertical line), have significant effects on the response and the more the standardized effect is, the more effective the factor will be. Pareto chart of moisture loss (Figure 4(a)) suggested that all the factors including seed moisture content, microwave power, air temperature and exposure time significantly affected the seed moisture loss, whereas, seed moisture content being most effective factor followed by exposure time. Additionally, microwave power had more contribution to moisture removal than the air temperature. According to Figure 4, (b) lentil seeds bed temperature was mostly affected by air temperature followed by microwave power, while other factors did not have any significant effect. Final lentil bed temperature is shown in Figure 5. The lentil seeds' bed temperature corresponded with increasing air temperature and microwave power. Its maximum value was 70°C at the power of 400 W for 18.5% MC and 500 W for 10.5% MC and air temperature of 60°C. Lentil seeds' surface temperatures were different from the bed temperature as the bed temperature was an average of seed surface and air temperature.

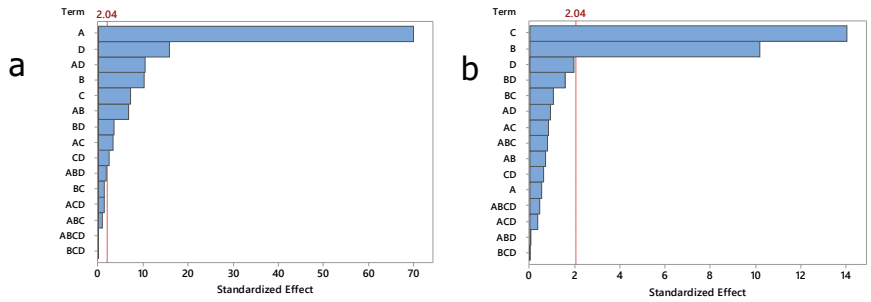

Figure 4 Pareto Chart of the standardized effects for (a) moisture loss and (b) bed temperature with a = 0.05; A = moisture content (10.5%, 18.5%), B = microwave power (0, 300, 400W for 18.5% MC and 0, 400, 500W for 10.5% MC), C = air temperature (50, 60°C), D = exposure time (5, 10 min)

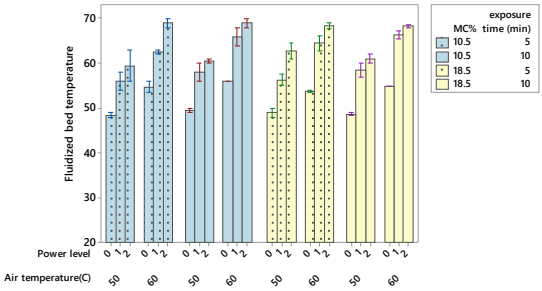

Figure 5 Final bed temperature at two set air temperatures of 50 and 60^{0}C, two exposure times of 5 and 10 min, microwave powers of 0, 300, 400W for 18.5% MC (power level of 0, 1, 2 respectively) and 0, 400, 500W for 10.5% MC (power level of 0, 1, 2 respectively); bars are SE from the mean; MC-moisture content

Effect of microwave power, air temperature and exposure time on seed viability and percentage of infected lentil seeds at each seed moisture content as a categorical factor
Generally, initial seed moisture content had by far the most influence on IS% using general full factorial regression with R^2 = 79%, F-Value = 42.62 and P-Value = 0.000. Therefore, statistical analysis for seed viability and IS% were carried out separately for each of moisture contents of 10.5% and 18.5%. The Pareto chart of standardized effects for germination percentage and infected seeds percentage counted after 4 days (IS%-4d) for treated lentil seeds at 18.5% MC is shown in Figure 6, (a) and (b) respectively. Both microwave power and air temperature had a significant effect on seed germination and IS%-4d. However, the effect of microwave power on IS%-4d was more than the effect of air temperature, while they affect seed germination almost equally. According to the results of main effect plots for the seeds at 18.5% MC, IS% decreased with increasing microwave power, air temperature and exposure time. Similarly, seed germination was negatively affected by increasing air temperature and microwave power from 300 to 400 W, while increasing microwave power from 0 to 300 had a positive effect on seed germination. For 10.5% MC, the general factorial regression did not provide a good fit and hence, no conclusion was derived about the effect of process parameters on IS% and seed viability.

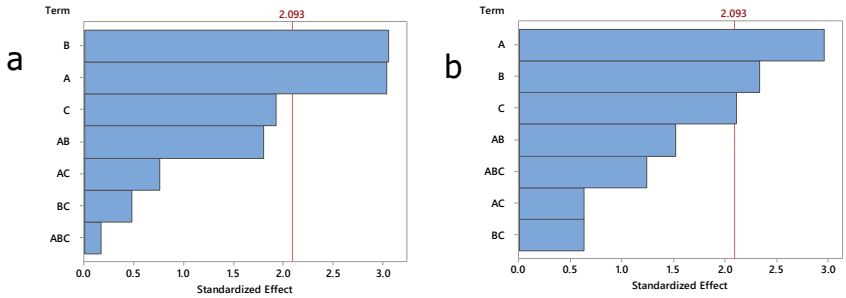

Figure 6 Pareto Chart of the standardized effects for (a) germination percentage and (b) infected seeds percentage counted after 4d of lentil seeds at 18.5% MC; A = microwave power (0, 300, 400 W), B = air set temperature (50, 60 °C), C = exposure time (5, 10 min); $\alpha = 0.05$

Table 1 represents the results of germination percentage (G%), infected seeds percentage counted after 4 days (IS% 4d) and 10 days (IS% 10d), and electrical conductivity of soaking water (EC) after microwave fluidized bed treatments of lentil seeds at two moisture contents of 10.5% and 18.5% with exposure times of 5 and 10 min. For seeds at 10.5% MC, none of the treatments affected germination, electrical conductivity or IS%, even at bed temperature of close to 70℃ (seed surface temperature of 75-80℃), which was obtained by microwave power of 500W and air temperature of 60℃ and exposure time of 10 min, while in the experiment with the water bath, the pathogen was eliminated at 75℃. The difference between these results could stem from the difference between relative humidity (RH) around the seeds at the time of treatments. Equilibrium RH around the seeds (water activity of the seeds) at 10.5% MC at 60℃ is between 60 to 75% (Menkov 2000) and as the water activity (and corresponding equilibrium RH) of the carbohydrate/protein rich foods increases by increasing temperature (Syamaladevi, Tang et al. 2016), RH of the seeds sealed in the bag in the water bath at 10.5% MC and temperature of 75℃ will be more than 60%. However, in microwave fluidized bed treatments, RH of air at the mentioned speed and temperature of 60 ℃ was measured as 11%, when ambient RH was about 50%, and this amount can be even less when the microwave is also used in combination with hot air. Therefore, the very low RH in the microwave fluidized bed could be the reason for increasing the lethal temperature of the seed pathogen. The other reason, which was mentioned by Syamaladevi, Tang et al. (2016), might be very fast desiccation of the pathogen in the microwave fluidized bed which could increase the pathogen's resistance to irreversible damage due to high temperatures.
Results for seeds with 18.5 % MC showed that by applying microwave power of 300W and air temperature of 60℃ for 10min, IS% counted after 10d (IS%-10d) reduced from 82% to 55% without any significant loss of viability. IS% -10d was reduced from 82% to 51.6% by applying microwave power of 400W and air temperature of 60℃ for 10 min; however, germination was also decreased from 95% to 60%. No significant change in electrical conductivity was observed as a result of these treatments. Here again, the difficulty of eliminating the seed pathogen could be related to the lower RH around the seeds. Nevertheless, it was more feasible to reduce the pathogen's load at higher MC of 18.5% than the dry seeds.
BGM pathogen growth on the seeds in BSM was evaluated on day 4 and 10, as after 4 days the pathogen mycelium, as well as the separated brown areas on BSM (tannin oxidation) were clearly discriminated and were comparable with the non-treated seeds. After 10 days, the mycelial growth was more and IS% in almost all the plates, including the control. The

difference between the treated and control samples was less than that on day 4. After 2 weeks of incubation, conidia were observed on the mycelium, however, the mycelium grew beyond the initially contaminated seeds and also infected healthy lentil seeds. Conclusively, reduction of IS% was considered after 4 (start of the growth) and 10 days (when healthy seeds could still be separated).

Conclusion

Microwave fluidized bed processing method was developed in this study to evaluate its potential for eradication of BGM pathogen from lentil seeds. The effect of process parameters on moisture loss, bed temperature, seed germination and infected seed percentage (IS%) were investigated. Most effective factors on moisture loss and bed temperature were seed moisture content and air temperature, respectively and microwave power was the most effective factor in IS% reduction. Air temperature and microwave power equally affected seed germination. Additionally, it was observed that eradication of BGM pathogen from lentil seeds in a microwave fluidized bed was more difficult than doing this in a sealed polyethylene bag in a water bath at the same temperature, which was concluded to be due to the difference in RH of the air around the seeds at the time of treatment. It is therefore recommended to investigate the effect of increasing RH of inlet air on seed pathogen reduction in future studies. BGM pathogen reduction, without significant seed viability loss in a microwave fluidized bed, was obtained by applying microwave power of 300W and set air temperature of 60°C on seeds with a moisture content of 18.5% for 10 min. This gave a 27% reduction in IS% from 82% to 55%. Therefore, microwave fluidized bed dryer can be considered as part of integrated disease management of BGM of lentil if the seed moisture content and process parameters are standardized for effective irradiation of the pathogen inoculum from the lentil seeds.

*Table 1 Germination percentage (G%), infected seeds percentage counted after 4 days (IS% 4d) and 10 days (IS% 10d), and electrical conductivity of soaking water (EC) after microwave treatments at different powers and air temperatures for 5 and 10 min; values with * are significantly different from their control based on Dunnett multiple comparison with* $\alpha = 0.05$ [1]

			Exposure time															
			5 min								10 min							
Moisture content% (wb)	MW power (W)	Set air temperature (°C)	G%	SD	IS% 4d	SD	IS% 10d	SD	EC (micro s/cm/g	SD	G%	SD	IS% 4d	SD	IS% 10d	SD	EC (micro s/cm/g	SD
18.5	0	50	95.0	7.1	67.5	3.5	80.0	7.1	96.0	9.3	86.7	14.4	48.3	18.9	68.3	14.4	95.3	1.6
		60	85.0	0.0	55.0	0.0	70.0	7.1	92.0	9.1	85.0	10.0	66.7	5.8	82.0	2.9	96.7	5.0
	300	50	96.7	2.9	53.3	16.0	75.0	18.0	85.4	5.4	92.5	3.5	47.5	3.5	72.5	3.5	93.0	0.4
		60	91.7	7.6	40.0	10.0	68.3	5.8	102.7	11.4	90.0	10.0	26.7*	7.6	55.0*	0.0	100.0	6.9
	400	50	95.0	0.0	57.5	3.5	77.5	3.5	96.9	2.3	82.5	3.5	42.5	3.5	60.0	0.0	91.5	7.4
		60	73.3*	12.6	40.0	22.9	71.6	12.6	103.8	0.8	60.0*	13.2	20*	18.0	51.6*	11.5	97.6	5.1
	control		95.0	5.0	58.3	10.4	82.0	12.6	98.7	6.7	95.0	5.0	58.3	10.4	82.0	12.6	98.7	6.7
10.5	0	50	80.0	14.1	50.0	7.1	85.0	0.0	82.6	2.3	92.5	3.5	65.0	21.2	95.0	7.1	84.6	3.9
		60	100.0	0.0	57.5	3.5	90.0	14.1	90.9	8.1	92.5	3.5	50.0	14.1	90.0	0.0	87.4	5.6
	400	50	90.0	0.0	42.5	10.6	92.5	3.5	84.3	6.0	85.0	7.1	45.0	28.3	90.0	0.0	80.7	9.9
		60	95.0	7.1	62.5	17.7	97.5	3.5	85.1	10.5	85.0	0.0	50.0	0.0	90.0	0.0	87.3	14.7
	500	50	87.5	3.5	67.5	10.6	90.0	0.0	86.1	8.9	95.0	0.0	67.5	10.6	92.5	10.6	87.2	5.4
		60	95.0	7.1	55.0	7.1	87.5	10.6	91.7	1.8	95.0	0.0	52.5	17.7	85.0	7.1	81.8	4.9
	control		97.5	3.5	80.0	14.1	100.0	0.0	87.5	15.5	97.5	3.5	80.0	14.1	100.0	0.0	87.5	15.5

[1] MW-microwave, G%-germination percentage, IS%- infected seed percentage, d - day, EC- electrical conductivity, SD- standard deviation

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