Effect of Heating on Bean Bruchid, *Callosobruchus Chinensis* L., (Coleoptera: Bruchidae) on Chickpea

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Abstract

The effect of heat treatment on different developmental stages of bean bruchid, *Callosobruchus chinensis* L., and chickpea seed viability and moisture content were evaluated. An electric solar simulator panel and an obtuse-base-angle box heater glued from inside with aluminium foil were used for heat treatment. The treatments were exposures to heat from 20-90 minutes at ten minutes interval. Heat treatment of *C. chinensis* infested chickpea for about an hour resulted in complete adult mortality and failure to lay egg. Eggs treated with heat did not survive exposures to heat for 40 minutes. Similarly, the eggs laid by adults that survived exposure to heat for 50 minutes failed to hatch. Moreover, larval instars and pupae did not tolerate heating for over 50 minutes. In general exposure of the various developmental stages to heat for about an hour in the aforementioned box heater raised the temperature within and between seeds to 60°C and more that provided effective control of the pest. Treatment of chickpea seeds with heat for up to 90 minutes had no adverse effect on seed moisture content and viability. Hence, chickpea seeds meant either for planting or consumption purpose could be protected from the pest by heat treatment.

Introduction

Grain legumes are important agricultural commodities in Ethiopia (Tsedeke *et al.* 1985). Despite their low yield per unit area due to biotic and abiotic constraints post harvest insect pests render severe damage often leading to complete loss. Bean bruchid, *Callosobruchus chinensis* L., is a major pest of stored pulses in Ethiopia (Walker and Boxall 1974, Tsedeke and Ampofo 1996). Chickpea *(Cicer aritenium* L.) is an important pulse crop under production (CSA 2005) that suffers much from this pest (Teshome 1994).

Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. However, development of resistance and pest resurgence (Subramanyam and Hagstrum 1995, Pedigo 1999), hazardous effect to other species and the environment (Kitch *et al.* 1992, Shaaya *et al.* 1997) and their high price are among the major factors limiting their use. These factors call for safer and cheaper alternatives to be integrated in the management of the pests.

To this end the use of extreme temperature is a technique that has been used successfully for many years against stored product pests. The potential of using solar energy in controlling storage insect pests through heating of grains in various types of solar heaters has been reported earlier. For instance solar heating of *Callosbrucus maculatus* (Fab.) infested cowpea seeds around noon in a solar heater made of black and clear polyethylene sheets trapped temperatures well in excess of 60 °c that is quite sufficient to kill the pest within 1 kg of cowpea seed (Murdock and Shade 1991). Kitch *et*

al. (1992) and Ntoukama *et al.* (1997) demonstrated larger capacity solar heaters (50 kg) effective in eliminating infestations of *C. maculatus* from cowpea seeds.

The aforementioned heaters, however, could disinfest small quantity of grain. Development of greater capacity solar heater that can disinfest large quantity in relatively shorter time is worth investigating. In view of this Mekasha (2004) compared various types of solar heaters and found that 118° obtuse-base-angle box heater glued from inside with aluminum foil trapped much solar energy. Treatment of *C. maculatus* infested adzuki bean seeds with heat in this heater completely controlled the pest in about 45 minutes and more solar exposure (Mekasha *et al.* 2006). This study, therefore, was initiated with the objectives of evaluating the potential of heating for the control of another storage pest of legume, *C. chinensis*, determine the optimum heat exposure time and effect of heat treatment on viability and moisture content of heat treated chickpea seeds.

Materials and Methods

General

The experiment was conducted at Debre Zeit Agricultural Research Center (38°58'N, 38°85'E, 1900 m a.s.l). An obtuse-base-angle box heater (Plate 1) was constructed from sheet metal with 118° base angle and 20 cm perpendicular height. The interior sides of the box heater that face the sun were glued with aluminum foil and plywood sheet was fixed to the exterior sides and the base using rivets to minimize loss of heat. An angled-iron frame was used to tightly fix the clear plastic sheet covering the box heater as a glazing material.

Intech Micro 2100-A 16' data logger was used to record temperatures sensed with 'Type J' thermocouples fitted to it and recordings were made at two minutes interval for 90 minutes with a computer using 'Microscan 2000 version 4' software. Thermocouple of 5 mm diameter was placed in the seed lot in between the seeds to sense the temperature between the seeds (hereafter referred to as 'between-seed temperature') and that of 1 mm diameter was inserted into the seed to sense the temperature inside (hereafter referred to as 'within seed temperature'). For this purpose the seeds were drilled with battery operated standard PBC drill (14,500 rpm) of 1mm diameter drill bit and the holes around the thermocouple were sealed with ground clay mixed with glue to avoid entrance of external air to the centre cavity of the seed. *Protimeter Grainmaster* moisture meter was used to measure seed moisture content. Petri dishes and filter paper were used for seed germination test.

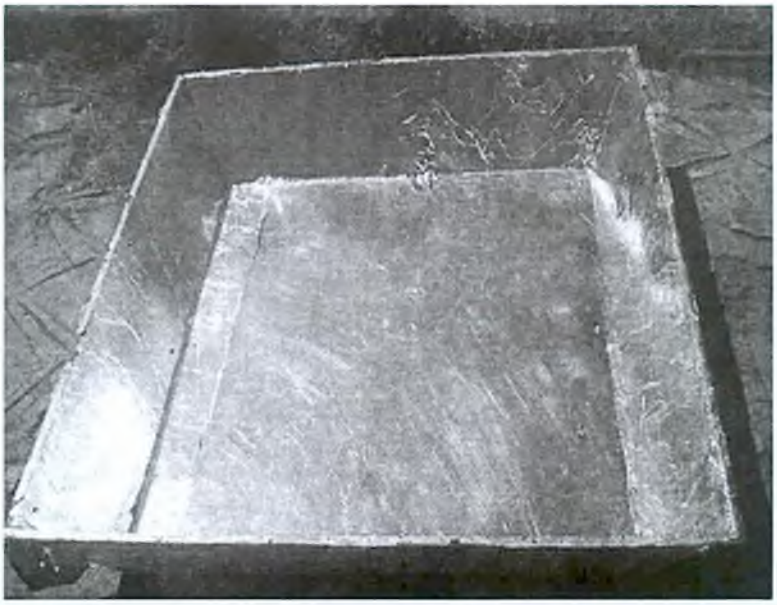

Plate 1. Obtuse-base-angle box heater glued from inside with aluminum foil

Chickpea seeds used were disinfested through heat treatment in an oven at 70°c for two hours. Stock cultures of C. *chinensis* were developed on the disinfested chickpea seeds from single pair of male and female adults. The cultures were maintained and allowed to multiply in the laboratory under room temperature, in aerated closed glass jars (3 cm diameter by a 3 cm height) to avoid cross infestation.

An electric power was used as a heat source for heat treatment using solar simulator panel, merely because it was difficult to get continuous 90 minutes bright sun shine during the study period due to intermittent cloud cover. Number of lamps and height of the solar simulator panel from the ground level that generated heat which simulated the heat trapped from natural solar energy (data from preliminary studies) was determined by correlating results of various combinations. Hence, heat generated by the solar simulator with eight lamps (500 volt, 250 watts) positioned at a height of 0.6 m from the ground level nearly perfectly simulated the natural condition with 98% correlation for both between-seed and within seed temperatures.

The subsequent experiments were laid in completely randomized design with three replications. The treatments were exposure to heat for 20, 30, 40, 50, 60, 70, 80, 90 minutes, and untreated check.

Heat treatment on adult mortality and oviposition

For each treatment 20 newly emerged adults were introduced to 100 g disinfested chickpea seeds in fabric bag and subjected to heat treatment immediately after introduction. After each treatment, the adults were separated from the seed by using a sieve of 5 mm mesh size. The adults then were introduced to 30 disinfested seeds and kept in the laboratory under room temperature in aerated glass jar. Dead and live insects were counted 24 hours after treatment and percentages of mortality were calculated. Eggs laid each day were counted to assess effect of heat treatment on oviposition.

Heat treatment on hatchability of eggs

A stock of newly emerged adults sufficient to lay eggs on 3 kg disinfested seeds was introduced to the seeds in fabric bags and allowed to stay for one day for oviposition. The adults were removed the next day by using a sieve of 5 mm mesh size. For each treatment, about 100 g seed with eggs were kept in small fabric bags and subjected to heat immediately after removal of the adults. Thirty randomly selected seeds to which treated eggs are attached were glued on label cards by facing the side of egg attachment upwards. These seeds were kept in the laboratory under room temperature to assess effect of heating on egg hatchability. Moreover, eggs laid by adults that survived short duration heat treatments from the preceding experiment were also kept in the laboratory in similar way to assess hatchability of eggs laid by heat treated adult.

Hatched and unhatched eggs were observed under a binocular microscope daily for a week to examine effect of heating on egg hatchability. Hatching eggs are distinguished by the activity of larva to bore in to the seed. Moreover, whitish color of hatched eggs as a result of the excreta packed in to the egg shell by the larvae upon hatching was used to distinguish them from unhatched eggs that remain dull white. Percent egg hatchability is determined by computing the percentile proportion of hatched eggs to the total number of eggs.

Heat treatment on larval instars and pupae

A stock of newly emerged adults sufficient to lay eggs on about 15 kg disinfested seeds was introduced to the seeds in fabric bags and allowed to stay for one day for oviposition. The seeds were kept in the laboratory under room temperature after separating the adults by using sieve of 5 mm mesh size. The result of the study on the biology of *C. chinensis* (Rahel 2008) showed that the majority of *C. chinensis* eggs developed to larval instar I, II, III, IV and pupal stage on day 6, 9, 11, 14 and 19 of their oviposition, respectively. Hence, about 100 g of seeds with eggs for each treatment were exposed to heat at these durations to treat the respective developmental stages. Thirty randomly selected seeds per treatment, each having single hatched egg, were kept in the laboratory under room temperature in cylinder glass jars covered with

perforated caps for aeration. The number of emerged adult bruchids was used to asses the effect of heat treatment on immature stages.

Determination of chickpea seed moisture and germination

The effect of heat treatment on moisture content of chickpea seed was determined by treating 300 grams of seeds with heat for different periods. *Protimeter Grainmaster* moisture meter was used to measure seed moisture content. Moisture contents of samples from the seed bulk were measured before heat treatment and the average value was considered as initial moisture content. Later moisture contents of the treated seeds were measured after the seeds were cooled for about an hour. The differences between the initial moisture content and those after treatment were considered as losses in seed moisture content due to the respective treatments.

Effect of heat treatment on seed germination (viability) was determined by testing 300 grams of seeds treated with heat for different periods. One hundred seeds from each treatment were spread on filter paper in Petri dishes and kept moist with distilled water for ten days. Number of germinated seeds was counted and the proportions to the total number of seeds were considered as percentage of seed germination.

Data analysis

Percentage proportions were calculated for data on adult mortality, egg hatchability and seed moisture content. Statistical Analysis Software (SAS) was used to analyze the data using analysis of variance (ANOVA) procedure. Tukey's Studentized Range Test was used for mean comparison.

Results

Effect of heat treatment on adult mortality and oviposition

Adults of *C. chinensis* were significantly affected when they were subjected to different regimes of heat treatment (Figure 1). Exposure to heat for 20, 30 and 40 minutes raised the between-seed temperature to 49.6°c, 52.9°c and 55.1°c that killed 86.7%, 81.7% and 95% of the adults, respectively. Extending the exposure time to 50 minutes was not different from the 40 minutes treatment in terms of adult mortality. However, adult mortality was complete (100%) at one hour exposure time. On the other hand, none of the adults that were not subjected to the heat (untreated check) died.

Heat treatment also affected oviposition, though the adults that survived the shorter duration heat treatment laid fewer eggs (Figure 2). The number of eggs laid during the first three days by adults exposed to heat for 20 minutes (23.7) and 30 minutes (22) were significantly different from those that were not heat treated (57.3). Moreover, significantly ($p<0.01$) low number of eggs (9.3 and 2.3) were laid by females exposed to heat for 40 and 50 minutes, respectively, compared to the short duration treatments.

Effect of heat treatment on egg hatchability

Hatchability of eggs significantly varied when they were exposed to different levels of heat and duration of exposure (Figure 3). Exposure of eggs to heat for 20 and 30 minutes raised the temperature between-seeds to 52.2°c and 54°c that resulted in 33.3% and 10% egg hatchability, respectively. These were significantly ($p\leq0.01$) lower than that of the untreated eggs (80%). Under further extension of the heat exposure periods none of the eggs were able to hatch.

Even though some adults have survived from heating for up to 50 minutes, the effect is expressed on hatchability of the eggs they laid. Adults that survived heating for 20 and 30 minutes produced eggs with low hatching rate of 15% and 10%, respectively. Significantly ($p<0.01$) lower percent hatchability (3%) was obtained from eggs laid by adults exposed to heat for 40 minutes. Eggs laid by adults treated for 50 minutes failed to hatch.

Effect of heat treatment on larval instars and pupae

Heat treatment affected the development of larvae and pupae (Figures 4) as indicated by the number of adults that emerged from 30 randomly assessed seeds. Exposure of first instar larvae for up to 30 minutes did not result in the emergence of significant number of adults compared to the untreated check. Heating this instar for 40 and 50 minutes that raised the within seed temperature to 58.3°c and 63.5°c significantly ($p\leq0.01$) reduced the number of adults emerged to 4 and 4.7,

respectively. Extended exposure of this instar resulted in complete kill. Similarly second and third instar larvae exposed to heat for 20 to 40 minutes resulted in significantly ($p \leq 0.01$) lower adult emergence compared to the untreated check. No adult emergence was recorded in the extended exposures of these instars.

Number of adults emerged (20.7) from fourth instar larvae heated for 20 minutes was not significantly different from the untreated check (24). Heating this instar for 30 and 40 minutes significantly ($p \leq 0.01$) reduced the number of adults emerged to 14.7 and 4.7, respectively. Similar to the results on younger instars no adult emergence was obtained from this instar when exposure times were extended to 50 minutes and more. On the other hand, pupae were found to be affected in shorter exposure period to heat compared to the larvae. Exposure of the pupae for 20 to 40 minutes resulted in significantly lower adult emergence compared to the untreated check. None of the pupae had developed to adulthood at 50 minutes exposure to heat where the temperature reached 64°C.

Effect of heat treatment on chickpea seed germination and moisture content

Heating chickpea seeds for up to 90 minutes that raised the between-seed temperature to as high as 62.3°c did not affect moisture content of the seed (Figure 5). Initial moisture content of the seeds before heat treatment was 11.3%. The various levels of heat treatment resulted in mean seed moisture content loss of only 0.1%. Moreover, exposure of chickpea seeds to heat for similar duration of time that generated as high as 67.9 °c heat did not show significant effect on its viability (Figure 6). Germination rate of not less than 96% was obtained under the various levels of heating.

Discussion

Heating *C. chinesis* infested chickpea for about an hour in an obtuse-base-angle box heater glued with aluminum foil from inside resulted in failure to lay eggs and complete kill of adults. Similar findings were reported by various workers. Kitch *et al.* (1992) reported that temperatures of at least 57°C were maintained for a minimum period of one hour that controlled *C. maculatus* oviposition on cowpea seeds. Solar heating of *C. maculatus* infested pigeon pea seeds in polyethylene bags trapped maximum temperature of 65°c (Chuahan and Ghaffa 2002). This resulted in complete death of the pest without laying eggs in all the solar-heated bags while considerable egg laying had taken place when the control treatment was examined five weeks after the treatment.

Bruchid eggs did not tolerate heat of about 56 °c obtained at 40 minutes exposure. Moreover the eggs laid by adults that survived exposure to heat for 50 minutes failed to hatch. This indicates that few adults that would survive heating for such a period could not continue their generation. Similarly, larval instars and pupae did not tolerate heating for over 50 minutes. This is in agreement with the findings by Murdock and Shade (1991) that revealed solar heating of *C. maculatus* infested cowpea seeds around noon using solar heater made of polyethylene sheet produced a temperature well in excess of 60°c and controlled adult emergence.

Temperatures below 5°c and above 45°c are lethal to storage insects; where at 50-60°c death occurs in minutes (Fields, 1992). In the current study temperatures of this range were trapped by the solar heater as early as 20-30 minutes exposure to heat. Extended exposures for 40-60 minutes resulted in temperatures well in excess of 60°c. This is largely due to the reflection of much light by aluminum, to the bottom of the heaters where the seeds were laid, because of its high reflectance, reflection coefficient of 0.9 (OSA, 1995). Such temperatures were responsible for the complete control of the various developmental stages of the pest. The heats used in this study were perfect simulation of that trapped from the natural solar energy around mid day (Rahel, 2008) using the same solar heater. Therefore, it could be extrapolated that solar heating of infested chickpea seeds using this solar heater for about one hour around mid day could be used to destroy the pest.

Fields (1992) also reported that change in lipids, rate imbalances, perturbation of ionic activities as well as desiccation have been proposed as possible mechanisms of death due to high temperatures. Phospholipid membranes become more fluid (destabilized) at high temperature. The nervous system, because it is so dependent upon membrane integrity, is thought to be especially sensitive to high temperatures. The structure of proteins is affected adversely by high temperatures. There is a

positive correlation between temperatures at which pyruvate kinase, a key enzyme in glycolysis, is inactivated. This enzyme is inactivated in 3 minute exposure to temperatures from 56 to 60°C, the same condition that kill most stored product insects. Enzymes are affected adversely at temperatures below which there is gross denaturation, and this causes rate imbalance in biochemical reactions.

Moreover, treatment of chickpea seed with heat did not affect its germination and moisture content. Thus, heat treatment of chickpea has no adverse effect as far as seed viability and moisture content is concerned. Therefore, chickpea seeds meant either for planting or food purpose could be protected from storage pests using heat treatment. Solar heating of pigeon pea and cowpea seeds to control C. *maculatus* also resulted in no adverse effect on their germination and moisture content (Chuahan and Ghaffa 2002; Murdock and Shade 1991). Mekasha (2004), however, did not recommend heat treatment for Adzuki bean seed meant for planting because of the considerable loss obtained in germination, though not significantly different from the untreated cheek. It is, therefore, important to assess the adverse effect of heating on such grain qualities as their sensitivity to high temperatures differs.

In general the results indicated the potential of using the free and safe solar energy for the control of storage pests. However, the amount of seed to be treated at a time and economic feasibility of the technology should be investigated for its practical application.

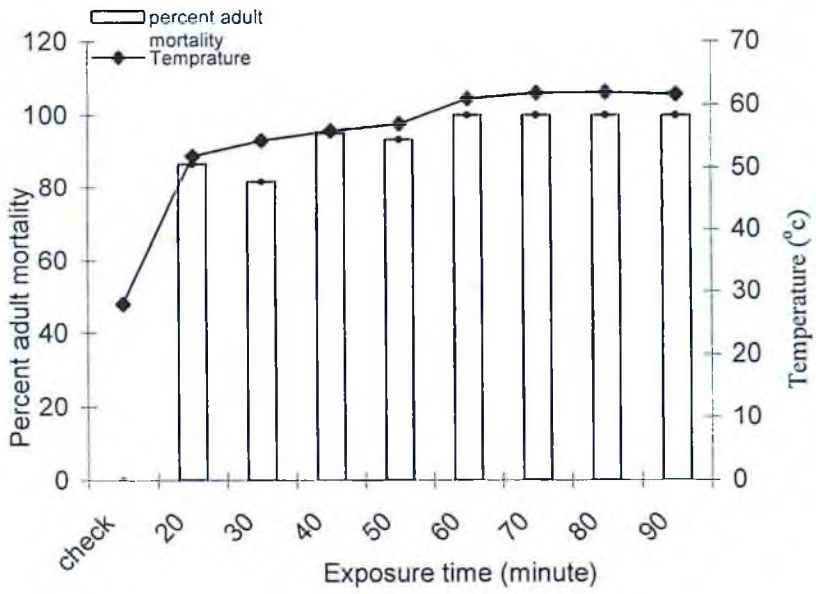

Figure 1. Mortality of adult *C. chinensis* as affected by heat treatment for different durations.

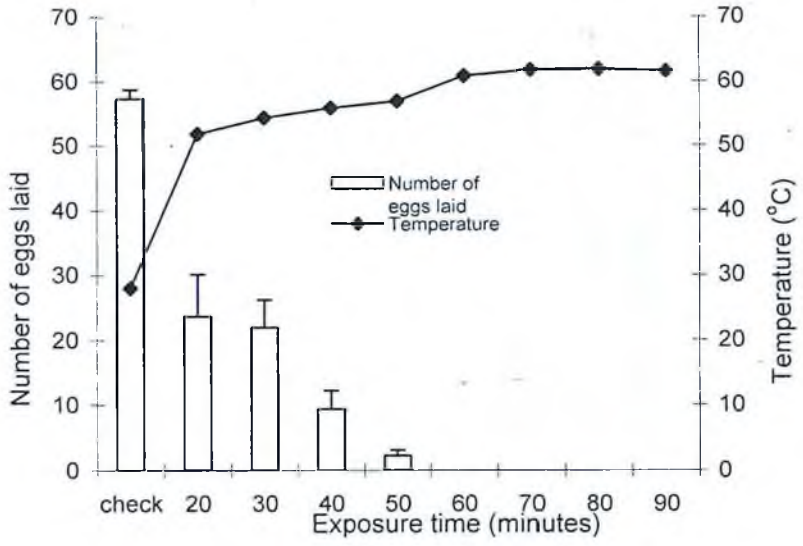

Figure 2. Number of eggs laid per heat treated female *C. chinensis* during the first three days (± standard error) after emergence

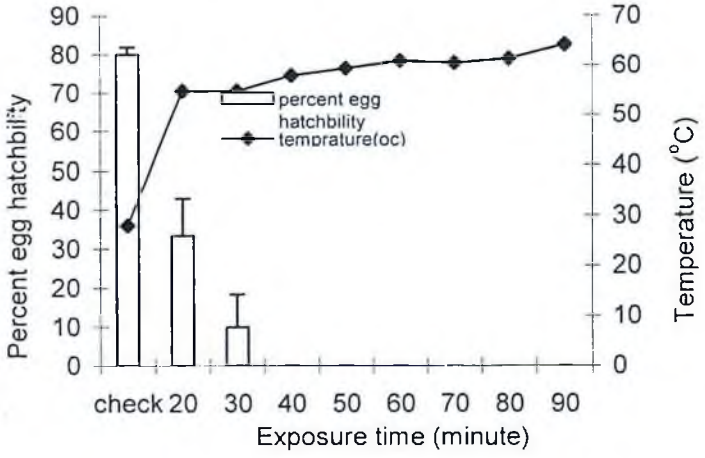

Figure 3. Hatchability (± standard error) of *C. chinensis* eggs as affected by heat treatment for different durations

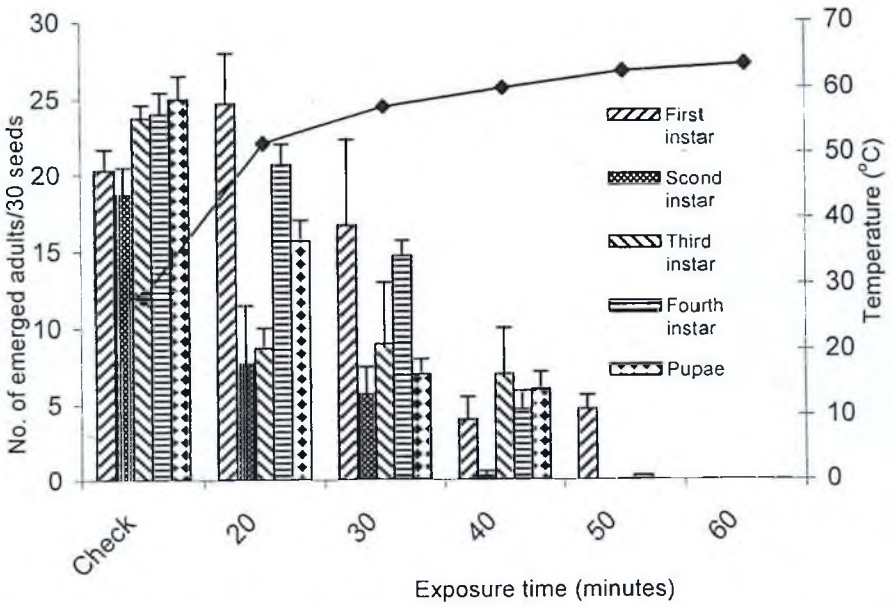

Figure 4. Number of *C. chinensis* adults emerged (± standard error) from larvae and pupae treated with heat for different durations

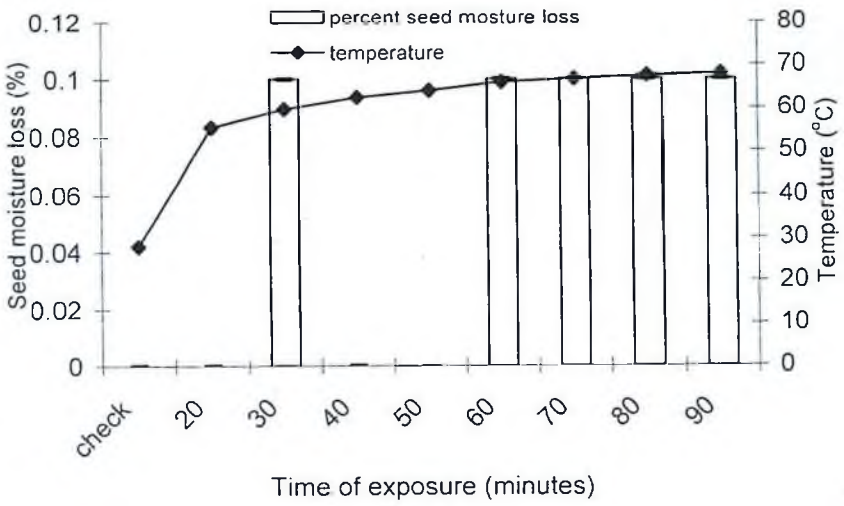

Figure 5. Loss in Chickpea seed moisture content due to heat treatment for different durations

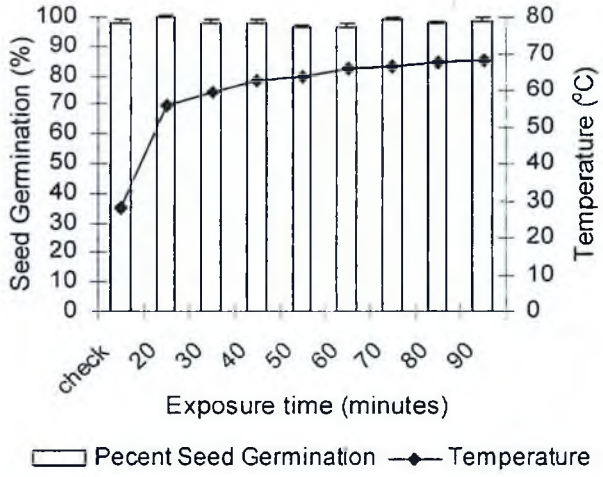

Figure 6. Germination of Chickpea seeds (± standard error) as affected by heat treatment for different durations

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