O.41 - Efficacy of various non-chemical methods against pulse beetle, *Callosobruchus maculatus* Fab.

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Abstract

Pulses (grain legumes) are excellent sources of proteins (20-40%), carbohydrates (50-60%) and are fairly good sources of thiamin, niacin, calcium and iron. In India, over 200 species of insects have been recorded infesting various pulses both in the field and storage. Among these, the pulse beetles *Callosobruchus* spp. are the major pests in storage. Generally, infestation starts in the field but population builds up in storage as the insect feeds inside the seed and emerges as an adult and causes secondary infestation inflicting heavy losses. The infested seeds are rendered unfit for human consumption as well as for sowing purposes. The most practical and successful method of controlling these insects are chemical methods. But due to environmental concerns there is a need to develop ecofriendly approaches such as physical methods. These comprise thermal treatments (both low and high temperature) and radiations (microwave, gamma rays and electron beam). Exposure of green gram seeds infested with different stages of *Callosobruchus maculatus* viz., egg, early larva, late larva and pupa to low temperatures at 20 ±1°C, 9±1°C and –14±1°C for 24h revealed that all the stages were highly sensitive to a temperature of –14±1°C and adult mortality at this temperature occurred within 12 min. Exposure of cowpea seeds infested with different stages of *C. maculatus* at 50°C for 2h, 4h and 6h revealed that all stages are sensitive to an exposure period of 6h at 50°C while complete mortality of adults at this temperature is achieved within 12 min. Exposure of cowpea and green gram seeds infested with different stages of *C. maculatus* to microwaves generated at frequency 2450 MHz for 70 seconds was effective against all the stages of pest. Exposure of adult *C. maculatus* to different doses of gamma rays revealed that a dose of 100Gy has a sterilizing effect on adults. These treatments have no significant effect on the germination of seeds. Further, the exploitation of electron beam for the control of this pest is in progress.

Pulses (grain legumes) are the second most important group of crops worldwide. Globally, 840 million people are undernourished mainly on account of inadequate intake of proteins, vitamins and minerals in their diets. Pulses are excellent sources of proteins (20-40%), carbohydrates (50-60%) and are fairly good sources of thiamin, niacin, calcium and iron. One of the major constraints in production of pulses are the insect pests which inflict severe losses both in the field and storage. In India, over 200 species of insects have been recorded infesting various pulses (CABI, 2007). Among these, pulse beetle, *C. maculatus* is a major pest that causes serious damage and is cosmopolitan.

In general, the damage starts in the field, where adult female lays eggs on the green pods. The grubs feed through the pod cover and remain concealed within the developing seed (Southgate, 1979). When such seeds are harvested and stored, the insect continues to feed as hidden infestation and emerges as an adult, causes secondary infestation and may cause total destruction within a period of 3-4 months and the grain/ seed is rendered unfit for human consumption as well as for sowing purposes (Singh and Jackai, 1985).

Conventional chemicals viz. grain protectants or fumigants have been extensively used all over the world to check infestation. However, presently they are being restricted globally because of the problems related to persistence of toxic residues in food grains, the development of insect resistance and adverse environmental impacts. Therefore, a need was felt to investigate the ecologically safe physical methods to control insect pests of pulses. The methods comprise thermal treatments (low and high temperature) and radiations (microwaves, gamma rays and electron beam). Pest control strategies are based on the understanding of the pest's biology, behaviour and physiological requirements. Temperature, is the most important factor of the environment, determining the rate of metabolism, growth, development, reproduction, general behaviour and distribution of insect pests. Each insect pest has a particular requirement of temperature to carry out its activities. Any temperature lower/ higher will affect insect's activities. Therefore, thermal treatments using temperatures higher and lower than the range have been exploited for pest control by various workers. Studies on thermal and low-temperature control of *C. maculatus* in USSR revealed that temperatures of 50, 55 and 60°C killed larvae and pupae in seeds within 125, 80 and 45 min, respectively, while a temperature not lower than 0°C killed all stages within 480 hrs and one of -10°C did so in 72 hrs.

The use of high frequency electrical energy in agriculture has been reviewed by several researchers. The *Triboilium confusum* and *Plodia interpunctella* exposed to 2450 MHz microradiation died as a result of their bodies reaching the lethal temperature of 80^{o}C for the most resistant life stage (Nelson, 1996). Microwave irradiation has also been found effective against the adults of *Sitophilus oryzae* infesting rice with no effect on seed germination (Bhalla *et al*., 2006) Gamma radiation at a range of 25-125Gy against 7-10 days old adults of saw-toothed grain beetle, *Oryzaephilus surinamensis* responded differently depending on dose level and all died at 125Gy. Irradiation at 120-1000Gy caused complete mortality of adults of *Tribolium confusum* in 12-15 days. The soft electron (electrons acceleration voltage 170Kev for 20 min (10kGy) treatment of seeds infested with different stages of *C. chinensis* adversely affect the longevity, fecundity and fertility of adults with no affect on the germination of adzuki bean seeds (Reddy *et al*., 2006).

In view of this, present studies are based on the efficacy of above-mentioned non-chemical methods for the control of pulse beetle, *C. maculatus*.

Material and methods

The pulse beetle, *C. maculatus,* was reared on green gram and cowpea seeds in the Entomology laboratory at the Division of Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi, India. The cultures were raised on healthy seeds using a single pair of freshly emerged adults and maintained under controlled conditions of temperature 29±1^{o}C and relative humidity 60±5% in the B.O.D. Incubator. Sub-culturing was done using a standard procedure. The insects were raised for about 6-8 generations before starting the experiments. Adults were sexed as per the identification characters described by Arora (1977). Infested seeds were prepared by releasing 0-1 day old adults (ten pairs for 100 seeds) for a period of 24h for oviposition/egg laying in culture jars. Experiments were conducted using seeds infested with different developmental stages of *C. maculatus*. As the development occurs inside the seed, test stages were prepared based on the number of days from oviposition. These comprised egg stage – one day after oviposition, early larva – 7 days after oviposition, late larva – 14 days after oviposition and pupal stage – 21 days after oviposition. The presence of different stages was also confirmed through soft X-rays generated at 22 kV, 3mA at a distance of 30cm for 30 seconds. In all the experiments one hundred infested seeds containing each developmental stage, each replicated thrice were used. The studies were based on the parameters of adult emergence and average development period.

Thermal treatments

Dry heat: One hundred infested seeds of each developmental stage in three replications were given dry heat treatment at 50±1^{o}C for exposure periods of 2h, 4h and 6h in the dry air oven. The treated seeds were then transferred to controlled conditions of temperature (29±1^{o}C) and relative humidity 60±5%. The infested seeds without treatment (control) also in 3 replications were kept in BOD under the same conditions. To study the effect of dry heat treatment at 50^{o}C on adult survival, 100 adults (1-2 days old) of *C. maculatus* in three replications were exposed for 10, 20, 30, 40, 50, 60 min and adult survival recorded. Healthy seeds of cowpea were also subjected to the same treatments as above and subjected to standard germination tests for testing the viability and vigour of seeds as per the ISTA Rules (ISTA, 1996).

Low temperature: Green gram seeds infested with each developmental stage of *C. maculatus* in three replications were exposed to low temperatures at 20 $\pm 1^oC$, 9 ± 1^oC and -14 ± 1^oC for 24hrs while control was kept at 29 ± 1^oC. To study the effect of low temperatures on adult survival, 100 adults (1-2 days old) of *C. maculatus* in three replicated trials were kept at -14 ± 1^oC for 10, 20, 30, 40, 50 and 60 min and adult survival was recorded after each exposure. Healthy seeds of green gram were also subjected to low temperature as above and subjected to standard seed germination test as per the ISTA Rules.

Radiations

Microwave radiations: One hundred seeds each of cowpea and green gram infested with each developmental stage of *C. maculatus* spread in single layer in petriplates in three replications were exposed to microwaves generated at 2450MHz for 0, 30, 35, 40,45, 50, 55, 60, 65 and 70 seconds.

Healthy seeds of cowpea and green gram were also treated as above and subjected to standard seed germination test as per the ISTA Rules (ISTA, 1996).

Gamma rays: One hundred seeds of green gram infested with each developmental stage in three replications were exposed to gamma rays (cobalt-60 source) at 100Gy, 200Gy, 300Gy, 400Gy and 500Gy. Also the healthy seeds of green gram were exposed to these doses and subjected to germination test as per the ISTA Rules.

Electron beam: One hundred seeds of green gram infested with each developmental stage spread in single layer in petriplates in three replications were exposed to electron beam (Beam energy=500 Kev, Beam current=12mA and speed of conveyor belt=10m/min.) at 170Gy, 340Gy, 510 Gy, 680Gy, 840Gy and 1000Gy. Also the healthy seeds of green gram were exposed to these doses and subjected to germination test as per the ISTA Rules.

Results

Thermal treatments

Dry Heat: Exposure of cowpea seeds infested with different stages of *C. maculatus* viz., egg, early larva, late larva and pupa at 50^oC for different durations viz., 2h, 4h, and 6h and normal temperature 28.5^oC revealed significant reduction in bruchid infestation in the heat-treated cowpea as compared to control. Adult survival decreased with increase in exposure period to dry heat after 4min upto 10 min. There was no survival of adults at 12 min exposure. The seed germination was slightly affected.

Low Temperature: Exposure of green gram seeds infested with different stages of *C. maculatus* viz., egg, early larva, late larva, pupa to low temperatures viz., $20\pm 1°C$, $9\pm 1°C$ and $-14\pm 1°C$ for 24h revealed that all the stages were highly sensitive to a temperature of $-14\pm 1°C$. Adult mortality at this temperature occurred within 12 min. The seed germination was slightly affected (Kumar and Bhalla, 2007).

Radiations

Microwave radiations (frequency-2450 MHz) against cowpea and green gram seeds artificially infested with different stages of *C. maculatus* viz., egg, early larva, late larva, and pupa exposed to microwaves for 30, 40, 50, 60, 70 and 80 sec revealed egg and early larva stages were very susceptible and no adult emergence occurred after exposure for 40 seconds. Pupa, i.e. hardiest stage, required 70 sec exposure to microwaves for complete inhibition of development. All the stages of *C. maculatus* were controlled by microwave radiation and the pest mortality increased with the increase in exposure period. Seed germination was not affected.

Gamma rays (Cobalt-60 source) Exposure of green gram seeds infested with different stages of *C. maculatus* with doses ranging from 100Gy to 500Gy revealed varying effects and that the 100Gy dose has a sterilising effect on the adult. The higher doses have varying effects and germination gets affected with the dose.

Electron Beam

Electron beam irradiation is effective against *C. maculatus* infesting green gram seeds. Further investigations are in progress.

These technologies with further studies can be used as eco-friendly means for the management of pulse beetle and can also be exploited for the control of other storage pests.

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