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Detection of pest-pathogen in forage legume seeds with X-Ray radiography

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Abstract
During storage, seeds are attacked by a number of agents including insect pests, which cause damage by infestation and results in loss of germination and vigour. Different techniques are used to separate healthy and infested seeds, most of which are complicated and time consuming. Soft x-ray radiography, a quick non-destructive and accurate method, was applied to determine the infestation in present study. Seeds of eleven range and cultivated fodder legumes were exposed to x-rays and the photographs of the internal structure of irradiated seeds were taken. Healthy seeds were seen as shining white whereas infested seeds were dull brown or dark coloured. For comparison, the infestation in the seeds was observed visually with naked eyes and also studied by lacto-phenol test. The maximum infested seeds (19.7%) were observed in Lucerne (*Medicago sativa*) followed by 19.3 per cent in Berseem (*Trifolium alexandrinum) var.* Wardan while minimum 7.7 per cent was observed in Berseem (*T. alexandrinum*) *var*. JHB-146 and 9.7% in Neel (*Indigofera astragalina*). Pest infestation in other forage legumes was between 11.0 to 18.0 per cent. Lacto phenol test provided almost similar results but visual observation with naked eyes, could identify only about half of the infestation in comparison to those recorded with x-ray radiography.

Key words: Forage seeds, Infestation, Insect-pest, Range legumes, X-ray radiography.

Introduction

Pest and pathogens are major problems in the forage crop seeds in post harvest storage condition. During storage, the seeds are infested /infected with various insects and microorganism. Sometimes, in large sized seeds or hard coated seeds of trees and shrubs, in spite of insect infestation, the embryo remain intact, resulting in no or less damage (Simak and Sahlen, 1981). In small size seeds, pest infestation is not visible with naked eye. Kew Seed Bank has been consistently examining its collections using x-ray technology since 1976. Initially x-ray technique was used in plant quarantine to detect the pests in incoming and outgoing seed material at NBPGR, New Delhi (Wadhi, 1983, Wadhi and Varma, 1971). Use of X-ray radiography was also applied for various other purposes in plant sciences. At IGFRI, x-ray studies were conducted for determining seed setting in grasses (Bahukhandi *et al.,* 2011). In the present study, seeds of 11 forage legumes were exposed to soft x-rays for determining insect pest infestation.

Materials and Methods

In the present study, physically cleaned seeds of *Clitoria ternatea* (cv *Aparajita*), *Cyamopsis tetragonoloba* (Guar or cluster bean), *Desmanthus virgatus* (Dashrath grass), *Indigofera astragalina* (Neel), *Leucaena leucocephala* (subabool), *Medicago sativa* (Lucerne or alfalfa), *Sesbania sesban* (Dhaincha), *Stylosanthus seabrana* (stylo) and three varieties of *Trifolium alexandrinum* (Berseem *var.* Wardan, BB-3 and JHB-146) were selected and subjected to x-ray radiation. Initially the seeds were carefully visualized with naked eye for infestation/ damage due to insect-pests. For x-ray radiography, the seeds were placed on glass plates (90 mm in diameter) in x-ray machine and exposed to x-ray beam for 11-15 seconds (depending upon x-rays to penetrate in seeds) at MX-20 at National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus New Delhi. The MX-20 x-ray machine is a self-calibrated machine and depending on seed structure it calibrates time of exposure (11-15 seconds in this case) to get sharp pictures of the seed. The x-ray images of the seeds were analyzed for the number of infested/ damaged and healthy seeds. In the present study, after exposure to x-rays, the pictures of infested or damaged and healthy seeds were seen on computer screen, their numbers were counted and observations were taken in three replicates of 100 seeds each for determining percent healthy and infested or damaged seeds. Another set of the seeds were studied using lacto phenol test (Sinha, 1993). Lacto phenol was prepared

by dissolving 20 g phenol crystals in 20ml lukewarm distilled water and mixed with 20 ml lactic acid and 10 ml glycerin. Seeds were submerged in test tubes containing lacto phenol and boiled for 10-20 minutes and left overnight. On next day, transparent seeds were washed with water and internal infestation was observed with the help of the microscope.

Results and Discussion

The picture of the seeds under x-ray exposure on the computer screen was analyzed for healthy/infected or infested condition. The seeds having transparent or white /clear light zone were found healthy and the seeds with dark, brownish colour or unclear mass were observed as damaged /infested. Sometimes scattered dark patches were seen in seeds, which were found partly damaged whereas the seeds looking as completely dark or brown/dull mass were found as completely damaged due to infestation of pathogens including insect pests. In present study, 11 samples of forage seeds were given x-ray doses to determine the infestation. The pictures of radiated seeds as shown on the screen by x-ray machine are presented in Fig. 1. The average seed damage due to pathogens including insect pests ranged between 7.7 to 19.7 per cent. Highest damage of seeds was observed In *Medicago sativa* (19.7%), followed by *Trifolium alexandrium* var. wardan (19.3%), *Leucaena leucocephala* (14.3%), *Sesbania sesban* and *Clitoria ternatea* (13.7%). Minimum damage due to pest infestation was recorded in *Trifolium alexandrium* var. JHB-146 (7.7%) and *Indigofera astragalina* (9.7%). Lacto phenol test confirmed the infestation observed with x-ray radiography. In lacto phenol treated seeds, the healthy seeds were transparent while infested or infected seeds were seen as dense or dark mass. In x-ray method, the damaged part/content of the seeds, due to pathogens was clearly seen as dark content and sometimes the insect pest was also seen inside the seeds without damaging the seed. In lacto phenol test, the insect can be seen only after heating *i.e.* killing of the seeds. In present study, the small sized seeds, seen as healthy with naked eye were found infested or damaged when observed with x-rays.

Insect damage is a serious problem in large seeded plant species while hard coated seeds are less susceptible to insect attack. Yang *et al.* (2006) observed presence of eggs of oriental fruit fly (*Bactrocera dorsalis*) and injuries caused due to insect using x-rays in fruits and seeds of different citrus, pome group and tomato. Young *et al.* (2007) did similar studies on seeds of *Brassica* and wheat to observe internal morphology, development, physiology and damage without harming and destroying the grains. Fesus (2008) also reported that in quarantine, inspection by x-ray method is reliable and is a rapid testing method for determining internal infestation of seeds.

It can be concluded that percent infestation in the seed lot of forage/ fodder seeds can be determined easily by using soft x-ray radiography method. For determining presence of hidden insect-pests, this technique is very useful particularly in small size seeds. Phenol test is also a traditional technique but takes a long time, kills the seeds and can not be applied in big seed lots. Similarly, visual observation of the infested seeds is not reliable and is time consuming process especially in small sized seeds. In the present study, the ratio of infested/ healthy seeds observed in X-ray radiography matched with the standard phenol test thereby confirming the accuracy of the test (Table-1). The study also revealed the accuracy of the X-ray radiography which is far superior to visual observation particularly in small seeded species where visual identification of internally damaged / infested seeds is difficult. Thus, with these results it can be concluded that the pest or pathogen infestation in the seed lots of forage/ fodder species can be determined with ease and with high accuracy by using x-ray radiography technique.

Table 1: Comparison of insect-pest infested seeds by soft x-ray radiography, phenol test and visual observation.

Forage Legume species	Infested seeds observed (%)		
	X-ray radio graphy	Lacto-phenol test	Visual observation
Clitoria ternatea	13.7	11.0	6.0
Cyamopsis tetragonoloba	12.0	10.7	7.0
Desmanthus virgatus	11.7	10.3	6.3
Indigofera astragalina	9.7	8.7	5.0
Leucaena leucocephala	14.3	12.3	11.0
Medicago sativa	19.7	17.7	10.3
Sesbania sesban	13.7	11.7	8.0
Stylosanthes seabrana	11.7	11.0	5.7
Trifolium alexandrinum cv wardan	19.3	17.7	10.3
T. alexandrinum cv BB-3	12.3	11.3	6.7
T. alexandrinum cv JHB-146	7.7	7.0	4.7

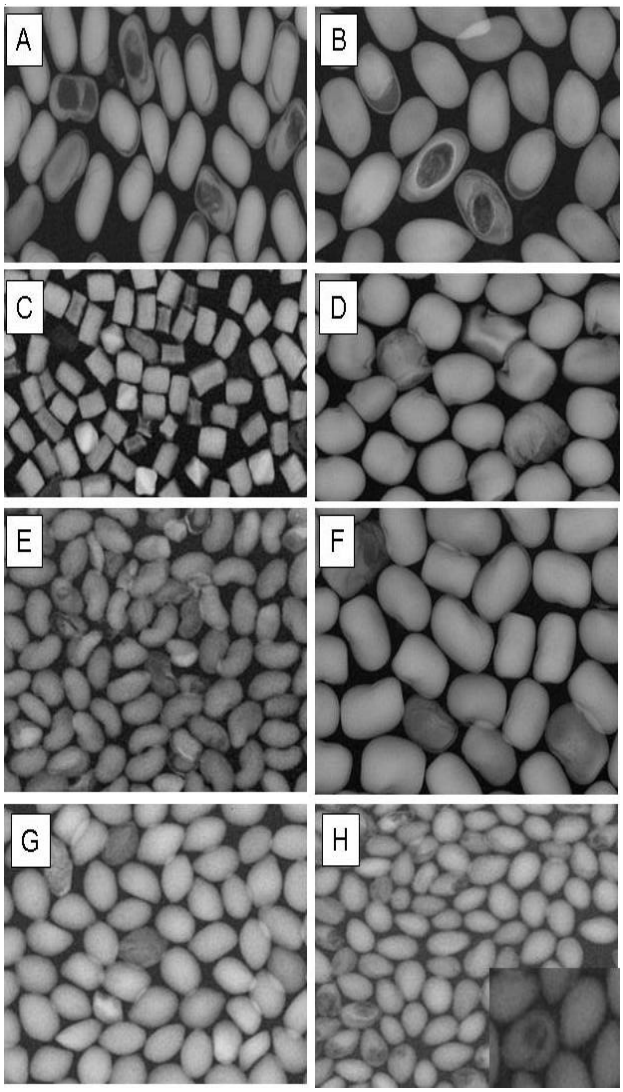

Figure 1: Forage legume seeds in x-rays exposure showing infested and healthy seeds: (A) *Sesbania sesban,* (B) *Leucaena leucocephala,* (C) *Indigofera astragalina,* (D) *Cymoposis tetragonoloba,* (E) *Medicago sativa,* (F) *Clitoria ternatea,* (G) *Desmanthus virgatus,* (H) *Trifolium alexandrinum* (Berseem-wardan).

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