CHAPTER V

5. Location of *Macrophomina phaseolina* in Mungbean and Blackgram Seed

5.1. An Overview

Seed transmission can be influenced by the amount and type of inocula, virulence, and its location in seeds (Agarwal and Sinclair, 1987). The location of the pathogen in seeds plays a vital role on disease severity and the firm establishment of the pathogen within the seed tissues as a pre-requisite for its effective transmission (Sharada and Shetty, 1987). True seed composes of four components, namely seed coat, cotyledon, radicle and plumule. Pathogen can be located in any component or all components of seed. It is possible that transfer of pathogen from seed to seedling and subsequent transmission of the pathogen occur in the seeds if the pathogen is located in the seed coat and upper layers of the cotyledons. Only cotyledon infection is more responsible for seedling mortality in the early period of growth than root infection (Raut, 1983). On the other hand, if cotyledons and embryos are heavily infected, the seed cannot germinate (Sharada and Shetty, 1987).

Location of *Macrophomina phaseolina* within the seed varies on the basis of seed structure. In pumpkin seed, this fungus was detected in all components of seed although the seed coat was found to be its major location (Sultana *et al.*, 1994). Gangopadhyay *et al.*, (1970) reported that *M. phaseolina* was found only in seed coat of soybean seed. Nevertheless, *M. phaseolina* was also detected in all parts of sannhemp seed and the maximum recovery was from the seed coat (Chaudhary and Pal, 1982).

To locate the site of infection of pathogens within the seed, the component plating technique is usually practiced (Raut, 1983). In this

technique, the various seed components are tested by the blotter method (ISTA, 1976).

Although the site of *M. phaseolina* was detected in several crop seeds but the location of this serious seed-borne pathogen has not been precisely investigated in mungbean and blackgram so far. Therefore, the present investigation was undertaken with the following objective:

- to ascertain the exact location of *M. phaseolina* within mungbean and blackgram seed.

5.2. Materials and Methods

5.2.1. Source of seed

Seed sample of mungbean and blackgram variety named as Chai Nat 60 and Uthong 2 respectively were obtained from Chai Nat Field Crops Research Center, Thailand. The seed samples of mungbean and blackgram found to be associated with seed-borne pathogen *M. phaseolina* by 29.75 and 24.00 percent respectively according to blotter method (ISTA, 1976).

5.2.2. Detection the location of *M. phaseolina*

The location of *M. phaseolina* within mungbean and blackgram seed was detected following the method described by Maden *et al.*, (1975). For determining the location of *M. phaseolina* in seed, at first one hundred seeds from each sample were separately soaked in sterilized water for five hours. Soaked seeds were dried on sterile blotter paper. The four components of each seed viz. seed coat, cotyledon, plumule and radicle were aseptically dissected and surfaced-sterilized with 10 percent solution of sodium hypochlorite for one minute. Then they were rinsed with sterilized distilled water for three to four times and dried on sterile blotter sheet.

The components of each seed were placed side by side on moist blotter paper (Whatman no.1) in 9cm-sterilized Petridishes (Plate 5.01 & 5.02). The boundary between adjacent seed components was marked clearly.

The Petridishes were incubated under 12 hours alternating cycles of NUV light and darkness for seven days as recommended by ISTA (1976). The presence of *M. phaseolina* (blackish microsclerotia and pycnidia) was examined by using stereo-binocular microscope in each incubated component of mungbean and blackgram seed.

5.3. Results

Results obtained from location detection of *M. phaseolina* in mungbean and blackgram seeds are presented in Table 5.01. The prevalence of *M. phaseolina* in seed coat was 28.0 and 23.0 percent in mungbean and blackgram respectively. The remaining seed components like cotyledon, radicle and plumule were free from *M. phaseolina* either in mungbean or in blackgram. On the seed coat of mungbean and blackgram, the fungus formed blakish microsclerotia and pycnidia (Plate 5.03 & 5.04).

5.4. Discussion

In the present study, seeds were soaked in sterilized water for easy dissection of various seed components. Raut (1983) proved the infection count of *M. phaseolina* in the various seed parts remained almost the same when the seeds were dissected either dry or after 12-14 hours soaking. This indicates that the infection does not spread from one part to the other during soaking.

From this investigation, it is revealed that in case of both mungbean and blackgram, *M. phaseolina* was exclusively located in the seed coat. Expression of the pathogen even after surface sterilization with sodium

Plate 5.01: Detection of site of *Macrophomina phaseolina* in different parts of mungbean seed. s- seed coat, c- cotyledon, pr- plumule & radicle.

Plate 5.02: Detection of site of *Macrophomina phaseolina* in different parts of blackgram seed by blotter method. s- seed coat, c- cotyledon, pr- plumule & radicle.

Table 5.01: Percentage of *Macrophomina phaseolina* incidence in different seed components of mungbean and blackgram.

Seed components	Percent incidence of *M. phaseolina*	
	mungbean	Blackgram
Seed coat	28.0	23.0
Cotyledon	0.0	0.0
Radicle	0.0	0.0
Plumule	0.0	0.0
LSD at 0.05	5.35	

Plate 5.03: Pycnidia and sclerotia of *Macrophomina phaseolina* were observed in the seed coat of mungbean under stereo-binocular microscope. P- Pycnidia.

Plate 5.04: Pycnidia and sclerotia of *Macrophomina phaseolina* were observed in the seed coat of blackgram under stereo-binocular microscope. P- Pycnidia

hypochlorite solution indicates the firm establishment of the fungus inside the seed coat. This finding was supported by Gangopadhyay *et al.*, (1970), where the seed coat was found to be the absolute site of *M. phaseolina* in soybean. Moreover, *M. phaseolina* has also been reported to be seed coat borne in several crops such as blackgram (Sharada and Shetty, 1987), sunflower (Raut, 1983), pumpkin (Sultana *et al.*, 1994), sannhemp (Chaudhary and Pal, 1982) and dry edible bean (Abawi and Pastor-Corrales, 1990).

From the present investigation, it can be concluded that the seed coat is exclusively the location of *M. phaseolina* in mungbean and blackgram seeds, which suggests that the pathogen is not internally transmitted. Based on this study it is inferred that controlling of this pathogen, surface seed treatment like coating of seeds with chemical fungicides or with biological antagonists in addition to hot water treatment might be effective.