SEED-BORNE INFECTION OF *ALTERNARIA BRASSICAE* IN INDIAN MUSTARD AND ITS ELIMINATION DURING STORAGE

A. S. CHAHAL

Department of Plant Breeding, Punjab Agricultural University, Ludhiana 141 004, *India*

ABSTRACT

Seed infection by *Alternaria brassicae* was present deep beneath the testa in all categories of seeds except brown coloured bold seeds. It was largely confined to discoloured seeds (84·0 to 92·0%). Brown coloured bold seeds had only 8·0% infestation on the testa and showed highest thousand seed weight (3·09 g) and oil content (43·83%) followed by bold gray coloured seeds (2·30 g and 43·50% respectively). Seed weight and oil content were markedly reduced in shrivelled seeds irrespective of their colour whereas gray colour of bold seeds did not interfere much with the oil contents. Seed infection by *A. brassicae* was found declining gradually during storage. The seed of var. RLM 198 (*Brassica juncea*) showing 32·0% and 42·5% infection in April 1979 and 1980 respectively became free of infection in September after storage at room temperature and at 35° C. Even the discoloured seeds having 84·0 to 92·0% infection in April were found completely free from it when tested in September after storage at room temperature. Thirty-five samples of rape and mustard seeds collected in the last week of September, 1979 from different locations in the Punjab also did not show any count of *A. brassicae*. The control of the disease in the field through fungicidal spray is however, very necessary in order to have better quality produce with bolder seeds.

INTRODUCTION

ALTERNARIA blight of rapeseed and mustard crops caused by *Alternaria brassicae* (Berk.) Sacc. is world-wide in occurrence. The pathogen attacks all above ground parts of the plants[1] and causes considerable reduction in yield[3]. It has been reported as seed-borne both externally and internally. Seed infection increases with the increase in infection of the pathogen on the pods and induces serious seed rot and seedling abnormalities when tested immediately after the harvest[2]. This investigation was conducted to obtain information regarding (i) the effect of seed-borne infection by *A. brassicae* on the health, colour, size and oil content of the seeds of mustard and (ii) its elimination during storage at different temperatures.

METHODS AND MATERIALS

Modified blotter technique (deep-freezing) recommended by Limonard[4] was followed to detect the presence of *A. brassicae* in mustard seeds throughout the present study. Four hundred seeds were used for each treatment.

Seed samples of mustard var. RLM 198 were collected after harvest in April 1979 from the crop severely infected by *A. brassicae* at the Nucleus Seed Production Farm of the Punjab Agricultural University at Ropar. From this mixture sample, four categories of seeds, *i.e.*, bold true-coloured (brown), bold discoloured (gray), shrivelled true coloured (brown) and shrivelled discoloured (gray) were separated with the help of forceps and examined in second fortnight of April, 1979 to detect the presence of *A. brassicae* in untreated and pre-treated (surface disinfested with 2·0% sodium hypochlorite for 5 minutes followed by one washing with sterilized distilled water) seeds.

Oil content was estimated by nuclear magnetic resonance spectrometer (Newport Analyser Model MK III A).

To study the effect of storage on the survival of *A. brassicae* in the seeds, a sample of mustard variety RLM 198 was stored at room temperature under laboratory conditions at the Punjab Agricultural University, Ludhiana, in the middle of April 1979 and tested at monthly intervals till the middle of September 1979. Four categories of seeds stored at same temperature conditions were also examined after five months in September. Twenty seed samples of mustard variety RLM 198 and 15 of unknown varieties of rapeseed-mustard collected randomly from the stores of Nuclear Seed Farm, Ropar and farmers' stores in the village of Bhatinda, Faridkot, and Sangrur districts of Punjab respectively in September and examined for *A. brassicae* counts.

Seed sample of RLM 198 were again collected after harvest in April 1980 from a severely infected crop by *A. brassicae* at the Research Farm of Punjab Agricultural University, Ludhiana, stored at four different temperatures, *i.e.*, at room temperature under laboratory conditions, in refrigerator and in incubators at 25° C and 35° C and examined for *A. brassicae* at monthly intervals till the middle of September 1980.

Mustard variety, RLM 514 sown at Oilseed Research Farm, Samala was sprayed thrice with Bordeaux

mixture (0·5%) at 15 days intervals starting when the crop was about 100 days old with a view to check the infection of *A. brassicae* from the pods. Unsprayed plots were also kept for comparison. The disease intensity was recorded on the pods before harvest on the basis of scale and formula used by Singh[6]. Seed samples (both sprayed and unsprayed treatments) collected after harvest were examined for the infection by *A. brassica* and its effect on the discolouration and shrivelling of the seeds.

Results

Discoloured (gray) seeds, irrespective of their size showed very high infection by *A. brassicae* (90 to 92% in bold discoloured and 84 to 90% in shrivelled discoloured seeds) and could not be eliminated by surface disinfestation. Mixture seed sample also showed fairly high infection in untreated (42%) and pre-treated (32%) conditions. The shrivelled but not discoloured seeds showed 18% infection in untreated and 14% in pre-treated seeds. The bold true-coloured (brown) seeds had mild infestation (8%) on the testa which was completely eliminated by surface disinfestation. Thousand grain weight and oil content were highest in bold true-coloured seeds (3·09 g and 43·83%) followed by that in bold discoloured seeds (2·3 g and 43·5%). Irrespective of their colour the shrivelled seeds were very poor in both grain weight and oil content. Mixture seed showing 1·83 g grain weight and 41·33% oil content was midway between the bold and shrivelled seeds.

Elimination of A. brassicae During Storage

The results of the experiments conducted for the two years (Table I) showed that the seed samples showing 30% and 42·5% infection counts of *A. brassicae* in April 1979 and 1980 respectively became free of it when stored at room temperature (29–38·5° C) and at 35° C in incubators for five months (from April to September). Reduction in seed infection was slow at 25° C and more slow in refrigerator wherein 11·1 and 25·2% seeds respectively showed *A. brassicae* infection in September. It has also been reported from Canada[5] that storing the rapeseeds for 6-8 months at 25° C reduces *A. brassicae* infestation by more than 50%.

The discoloured seeds showing 84 to 92% infection in April and twenty and fifteen samples collected respectively from the stores of Nuclear Seed Farm, Ropar and from the stores of farmers in the village, of different districts of Punjab in the beginning of September were also found to be free of *A. brassicae* when tested in the middle of September.

It was seen that the control of *Alternaria brassicae* from the pods of mustard with Bordeaux mixture (0·5%) helped not only in increasing the grain yield from 916·7 kg/ha (in unsprayed) to 1121·6 kg/ha but also reduced seed infection counts from 50% in unsprayed to 4% in sprayed treatment. Healthier pods also resulted in considerable reduction in discolouration and shrivelling of the seeds and increased the ratio of bold true-coloured seeds from 58·4% in unsprayed to 95·4% in sprayed treatment.

Conclusion

From the present findings, it may be concluded that *Alternaria brassicae* survives beneath the testa and

Table I

Effect of storage on seed infection by A. brassicae *in mustard (RLM 198) when stored at different temperatures from April to September 1979 and 1980*

Month	Per cent seed infection				
	Room Temperature*		35° C	25° C	Refrigerator
	1979	1980			
April	30	42·5 (—)			
May	24	36·0 (—)	28·5	30·0	40·3
June	18	22·6 (38·5/30·5)	23·8	26·2	29·4
July	10	2·2 (38·0/29·0)	1·8	24·5	29·0
August	—	0·0 (33·0/29·5)	0·0	17·3	27·6
September	0	0·0 (34·5/29·5)	0·0	11·1	25·2

* Temperature given in parentheses is the Maximum/Minimum of previous 30 days of middle of the month which it is mentioned. Room temperature was not recorded during 1979.

causes severe discolouration and shrivelling of the mustard seeds. Shrivelled seeds lose their weight and oil content considerably. Discolouration without shrivelling though reduces grain weight but does not interfere much with the oil content.

Rape and mustard seeds kept for five months (from middle of April to middle of September) at room temperature ranging from 29 to 38·5° C (natural conditions) or at 35° C or in the natural storage conditions of Punjab became free of *A. brassicae* infection before the start of sowing season. *Alternaria brassicae* although gets eliminated from the seeds yet leaves its bad effects like discolouration and shrivelling. To overcome this, control of the disease in the field especially on the pod stage is very essential because it directly helps to get a healthy and better quality produce.

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THE EFFECT OF SHORT-TERM TREATMENT OF METHALLIBURE [ICI COMPOUND 33, 828] ON THE HISTOMORPHOLOGICAL AND ENZYMATIC ASPECTS OF TESTIS AND THUMB PAD OF TOAD, *BUFO MELANOSTICTUS*

R. D. KANAMADI AND S. K. SAIDAPUR
Department of Zoology, Karnatak University, Dharwar 580 003, *India*

ABSTRACT

The short-term treatment with low dose of methallibure (ICI compound 33, 828) to adult male toads (*Bufo melanostictus*) had no effect on testicular histomorphology and spermatogenesis. However, the Leydig cell nuclear diameter as well as 3β-HSDH and G-6-PDH enzyme activities decreased markedly. This decrease in steroidogenic activity of the Leydig cells was correlated with the regression of thumb pads, which are known to be androgen dependent sex structures in male Anura.

THE antigonadotropic effect of methallibure is well established in mammals[1], but comparative studies on lower vertebrates are scarce[2–7]. The present work was undertaken to investigate the effects of low dose of methallibure on spermatogenesis, the steroidogenic activity of the Leydig cells, and the androgen dependent thumb pads of toads, *Bufo melanostictus*.

Adult male *B. melanostictus* obtained during the breeding season (July) from the surrounding areas of Dharwar were used after five days of acclimatization to the laboratory conditions. The first group specimens (10) were injected (ip) with saline to serve as the controls. The second group specimens (10) were injected (ip) with saline suspension of methallibure, biweekly for 20 days. The total dose being 5·25 mg for each of the experimental toad (750 μg/injection). All toads were autopsied one day after the last injection. The relative testis weights were recorded and representative pieces of testes and thumb pads were fixed in Bouin's fluid for histological and histometric studies[8]. The remaining pieces of testes were used for the histochemistry of Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) as described earlier[9].

It is evident from Table I that there is no appreciable effect on the average testis-weight, testis diameter and tubule diameter due to short-term treatment with methallibure. Similarly, no marked alteration in the spermatogenetic activity was observed (Table II). However, Leydig cell nuclear diameter decreased (Table I) significantly ($p \lesssim 0{\cdot}01$). Similarly, in treated toads the Leydig cell 3β-HSDH and G-6-PDH activities decreased (Table I) compared to the controls. The height of epidermis and glandualar epithelium of thumb pads also decreased significantly (Table III). The thumb pad epidermis was less papillate and the mucous glands were atrophic in the treated specimens. The present findings are thus in conformity with those reported earlier on other species[1–5,7], wherein methallibure was found to cause regression of the secondary