

Role of Seed Treatment in the Management of Bacterial Blight of Cotton

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Bacterial blight of cotton is known to be caused by a bacterium *Xanthomonas campestris* pv. *malvacearum*. It is a most serious problem of cotton cultivation both in India and abroad. Seed borne inoculum allows long-range dispersal, introduces the pathogen or new races of the pathogen into new areas, new countries and new fields. Good quality seed with low incidence of bacterial infection may be produced when seed is subjected to suitable treatment. Seed treatment helps in the management of the pathogen which has penetrated to the living cells of the seed. In the present study, cotton seeds of different varieties such as DCH-32, DHB-105, HYC-VLC and LRA-5166 were subjected to physical, chemical and biological treatments to reduce the incidence of bacterial blight of cotton. Cotton seeds were subjected to treatment with hot water at 50, 60 and 70°C, concentrated sulphuric acid, sodium hypochlorite, K-cycline and *Pseudomonas fluorescens* to test their efficacy in the management of the blight disease.

Cotton seed samples of varieties viz., DCH-32, DHB-105, HYC-VLC and LRA-5166 collected from KSSC at Bangalore were subjected to seed treatment with hot water, concentrated sulphuric acid, sodium hypochlorite solution, K-cycline and *Pseudomonas fluorescens*.

In case of hot water treatment 1200 seeds of each variety were separately suspended in a series of beakers containing hot water and maintained at 50°, 60° and 70° C for a duration of 30 minutes and then were air dried under shade for an hour. In sulphuric acid treatment, seeds of the above mentioned varieties were suspended in concentrated acid at

the rate of 150 ml /kg of seeds for 2-3 minutes, subsequently neutralized with lime water (20g lime/litre) and then the seeds were washed 4-5 times with distilled water. Such seeds were then air dried and subjected to modified germination test. In the other set, a surface disinfectant, sodium hypochlorite (NaOCl) was used to treat the seeds. For this purpose the seeds of each above mentioned varieties were soaked in NaOCl solution of 2 per cent available chlorine for 15 minutes at 50° C. After treatment the NaOCl solution was poured off immediately and the seeds were rinsed twice with 100 ml sterile distilled water for 3-4 minutes. Treated seeds were shade dried and subjected to modified germination test. In K-cycline treatment, seeds of each variety were soaked in the solution of K-cycline at 0.1g/500 ml of distilled water for overnight and air dried under shade, finally subjected to modified germination test. An antagonistic strain of *Pseudomonas fluorescens* was isolated from the native soil and the same was maintained on King's B medium. The bacterium was mass multiplied on the KB broth in a series of 500 ml conical flask. After 48 hours of incubation, culture broth was centrifuged at 10,000 rpm for 10 minutes. The pellets were suspended in sterilized distilled water and used to treat the seeds of each variety at the rate of 500ml/100g of seeds for a duration of 24 hours as described by Ravindran and Vidyasekaran [1]. The treated seeds were dried under shade and subjected to modified germination test.

Seeds in both the treated and untreated sets were subjected to modified germination test, in which the seeds soaked in sterilized distilled water for a

period of 16-18 h and sown in sterilized sand beds in plastic trays. Further, these samples were incubated for a period of 12 days under 12h/12h light and darkness at $22\pm 2^\circ$ C. On 12th day of incubation, the entire setup were examined for the occurrence of symptoms on the seedlings. Total number of emerged seedlings were also recorded. Based on the appearance of circular water soaked, translucent lesions on the cotyledonary leaves, the disease incidence was confirmed, per cent disease incidence was calculated and the results were tabulated, accordingly. The seeds subjected to soaking in distilled water maintained under similar conditions were served as corresponding controls.

The data on the treatment of cotton seeds with hot water, concentrated sulphuric acid, sodium hypochlorite, K-cycline and *Pseudomonas fluorescens* indicated the varied response with respect to the incidence of disease (Table 1). In case of hot water treatment, highest germination was observed at 60°C followed by 50°C and 70°C and the disease incidence was found to be reduced in 60°C than in 70°C and 50°C . In DCH-32, DHB-105, HYC-VLC and LRA-5166, among the control and treatment there was difference in the disease incidence. Similarly hot water treatment indicated the varied response at different temperature conditions (Table 1). In concentrated sulphuric acid treatment, highest disease incidence was noticed in LRA-5166 followed by HYC-VLC, DHB-105 and DCH-32. However, highest germination was recorded in DCH-32 followed by HYC-VLC, DHB-105 and LRA-5166. In DCH-32, DHB-105, HYC-VLC and LRA-5166, the disease incidence was 4.5, 3, 3.9 and 5.92 per cent. In sodium hypochlorite treatment, highest germination was observed in HYC-VLC, followed by DCH-32, DHB-105 and LRA-5166. The highest disease incidence was in LRA-5166 followed by HYC-VLC, DCH-32 and DHB-105. In DCH-32, DHB-105, HYC-VLC and LRA-5166, the difference among the data between control and treatment with respect to germination and disease incidence was 0.7, 2.05, 1.2, 2.2, and 5.94, 6.45, 7.32, 5.05 per cent, respectively. In K-cycline treatment, highest disease incidence was observed in LRA-5166 followed by HYC-VLC, DCH-32 and DHB-105. Enhanced germination was recorded in HYC-VLC followed by DCH-32, DHB-105 and LRA-5166. The difference between control and treatment with respect to germination and disease was 0.75, 2.1, 0.49, 2.2 per cent and 0.75, 2.1, 0.49, 2.2 per cent, respectively. *Pseudomonas fluorescens* treatment also showed the reduced disease incidence in LRA-5166 than any other varieties. In DCH-32, DHB-105, HYC-VLC and

LRA-5166, the difference among control and treatment with respect to disease incidence was 5.25, 5.45, 5.75 and 2.15 per cent. The germination was also varied by 5.75, 8.65, 5.6, and 1.05 per cent.

In all the treatment, in contrast to hybrids the susceptible variety LRA-5166 showed highest disease incidence, because of its susceptible nature. Thermotherapy has been applied to a number of bacterial diseases in different plant parts with reasonable success, including true seeds and plantlets or cuttings [2, 3, 4]. Hot water treatment is cheap and economic, it reduces the cost and also ecofriendly, it minimizes the hazardous effects of various chemicals on environment, which leads to pollution. It kills most of the disease causing organisms on or within the seeds. Improper treatment can cause seed injury. In cotton *X. axonopodis* pv. *malvacearum* infected seeds soaked in hot water for 10 min at 56°C eliminated the pathogen without affecting seed germination [5, 6]. Chupp and Sheriff [7] recommended the hot water treatment of several vegetable seeds against bacterial diseases. This concept remains quite parallel with the present findings. In India, both fuzzy and acid delinted seeds of cotton are being used for sowing purposes. Fuzz located bacterial infections, especially blight pathogen, can be minimized by using delinted seeds. Under Indian conditions, the use of concentrated sulphuric acid has certain limitations. However, internally infected seeds are the frequent source of primary inoculum for epidemics of bacterial blight of cotton which can not be avoided by acid delinting alone [8]. The increase in the germination due to acid delinting may be attributed to the removal of thick fuzz which mostly reduces the water absorption capacity of the seed [9]. Sowing of such seeds ensures better germination due to softening of seed testa. Eradication of *X. campestris* pv. *zinniae* from *Zinnia* seeds was obtained by soaking seeds for 30 min in 10-500ppm sodium hypochlorite (NaOCl), whereas soaking in streptomycin solution caused phytotoxicity and hot water treatment for 30 min at 53°C had negative effect on germination [10]. In the present investigation, in contrast to sodium hypochlorite, concentrated sulphuric acid treatment proved better to treat the cotton seeds. K-cycline is an antibacterial antibiotic, it is a yellow coloured free flowing powder containing streptomycin sulphate and tetracycline hydrochloride in the ratio of 9:1, is readily absorbed by the plant tissues. Hot water with chemicals is better for growth of plants against the

Table 1. Effect of different treatments on germination and bacterial blight disease in different cotton varieties

Treatment	Response of different varieties of cotton seeds to seed treatment in relation to seed germination and disease incidence															
	Seed germination (%)						Disease incidence (%)									
	DCH-32		DHB-105		HYC-VLC		LRA-5166		DCH-32		DHB-105		HYC-VLC		LRA-5166	
C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	
Hot water (°C)50	61±6.01 ^c	75±1.1 ^a	65±5.45 ^b	86±1.4 ^a	68±5.6 ^a	73±1.3 ^a	62±0.85 ^{bc}	51±1.2 ^b	7±1.2 ^b	2±0.40 ^b	8±1.6 ^c	2±0.2 ^a	9±1.9 ^a	2±0.5 ^b	11±1.5 ^a	4±0.2 ^a
60	61±6.01 ^c	89±1.7 ^a	65±5.45 ^b	86±1.4 ^a	68±5.6 ^a	89±1.4 ^a	62±0.85 ^{bc}	74±0.8 ^b	7±1.2 ^b	2±0.4 ^a	8±1.6 ^c	2±0.2 ^a	9±1.9 ^a	2±0.4 ^a	11±1.5 ^a	2±0.4 ^a
70	61±6.01 ^c	51±0.7 ^c	65±5.45 ^b	57±1.25 ^b	68±5.6 ^a	66±1.5 ^a	62±0.85 ^{bc}	50±1.9 ^a	7±1.1 ^b	1±0.4 ^b	8±1.6 ^c	1±0.47 ^b	9±1.9 ^a	3±0.4 ^a	11±1.5 ^a	2±0.2 ^a
Sulphuric acid	73±1.4 ^a	92±0.6 ^a	72±0.8 ^a	89±1.2 ^a	64±0.64 ^b	90±0.2 ^a	59±1.10 ^c	83±3.5 ^b	8±0.2 ^b	4±0 ^a	7±0.25 ^b	4±0.5 ^a	8±0.70 ^b	5±0.28 ^b	11±0.64 ^a	5±0.47 ^a
Sodium hypochlorite	77±6.01 ^a	84±5.2 ^{ab}	75±5.45 ^b	82±4.4 ^{ab}	78±5.6 ^a	88±2.17 ^a	82±0.85 ^b	75±.30 ^b	8±1.1 ^a	7±1.4 ^a	7±1.6 ^a	6±1.3 ^a	8±1.9 ^a	7±1.7 ^a	11±1.5 ^a	9±1.3 ^a
K-cycline	76±1.2 ^a	84±5.2 ^b	77±2.3 ^a	82±4.4 ^{ab}	78±5.63 ^a	88±2.1 ^{ab}	82±0.85 ^b	75±3.3 ^a	8±1.19 ^a	7±1.4 ^a	8±1.65 ^b	6±1.3 ^a	8±1.19 ^a	7±1.7 ^a	11±1.5 ^a	9±1.3 ^a
<i>Pseudomonas fluorescens</i>	76±1.2 ^a	92±0.9 ^a	77±2.3 ^a	87±0.7 ^a	83±2.5 ^a	91±0.6 ^a	75±3.4 ^a	77±0.6 ^c	8±0.5 ^b	3±0.25 ^b	9±0.4 ^b	3±0.45 ^b	9±1.0 ^b	4±0.25 ^b	9±0.9 ^a	8±0.25 ^b

Data based on 1200 seeds

According to DMRT, data represented by similar superscripts did not remain significantly different at P ≥ 0.05

C = Data with respect to control set

T = Data with respect to treatment set

bacterial infections. Similar effects were also reported with respect to black-rot of cauliflower caused by *X. campestris* pv. *campestris*, in which there was reduction of disease by more than 94% [11]. Biological seed treatment control the seed pests by parasitizing the pathogen. A cotton phylloplane bacteria (plb) belongs to genus *Pseudomonas* is being used for intensive studies on the basis of its *in vitro* antagonistic activity against most of the predominant races of *Xanthomonas axonopodis* pv. *malvacearum* [12]. Hence, the present findings inferred the integration of promising treatment which will definitely help to overcome the blight disease of cotton.

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