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INDUCTION OF SYSTEMIC RESISTANCE IN COTTON AGAINST BACTERIAL BLIGHT AND ITS EFFECT ON YIELD

Muhammad Atiq[1,] *, Sidra Arooj[1], Nasir Ahmed Rajput[1], Muhammad Rizwan Bashir[2], Nazir Javed[1], Ehteshamul Haq[3], Waseem Abbas[4] and Babar Khan[1]

[1]*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.*
[2]*Oil seed Research Institute, Ayyub Agriculture Research Institute, Faisalabad, Pakistan.*
[3]*Plant Pathology Research Institute, Ayyub Agriculture Research Institute, Faisalabad, Pakistan.*
[4]*Vegetable Research Institute, Ayyub Agriculture Research Institute, Faisalabad, Pakistan.*
[*]Corresponding author Email: dratiqpp@gmail.com

ABSTRACT

Bacterial blight (*Xanthomonas campestris* pv. *malvacearum*) of cotton is an important severe disease occurring in all cotton growing areas of Pakistan. Resistance to it may be systemically induced when plant defenses are preconditioned through prior infection or treatment against subsequent challenge by the pathogen. Here, we conducted field trial to induce systemic resistance against the bacterial blight of cotton and their effect on cotton yield. The disease was artificially inoculated with 10^9 CFU/mL by spraying on lower surface of healthy leaves. The experiment had five plant activators as treatments which were salicylic acid, K_2HPO_4, KH_2PO_4, citric acid and benzoic acid with three concentrations (1, 2 and 3%). Results showed that among these five plant activators, salicylic acid at 3% concentration had the best systemic acquired resistance and high yield followed by citric acid.

Keywords: Salicylic acid, K_2HPO_4, KH_2PO_4 Citric acid and Benzoic acid

INTRODUCTION

Cotton (*Gossypium* spp.) is leading cash crop all over the world. In Pakistan, its cultivated area grown over 15% with the 8.6% value added in agriculture and GDP about 1.9% (Anonymous, 2013). This triggers various business activities among factories and enterprises. Handling and processing of cotton after it leaves the farm provides more business activities described by (Islam *et al.,* 2003).

A number of biotic and abiotic stresses are constantly exposed by plants throughout their life cycles. These stresses adversely affect plant growth and reproduction, resulting in considerable yield losses annually worldwide (Helliwell *et al.,* 2013). However, these factors effect the cotton production and reduce the yield but bacterial blight is among the utmost important diseases affecting cotton crop initiated by *Xanthomonas compestris* pv. *Malvacearum* which infects the aerial portions of the host (Saha *et al*., 2001). Twigs rotting, vein blight, angular leaf spot, boll rotting and black arm are distinguishing signs of bacterial leaf blight described by (Ramapandu *et al.,* 1979; Inner, 1970; Watkins, 1981). Losses contributed by this disease were about 10 to 30 % among various cultivars (Kalpana *et al*., 2004). Fiber also becomes weak and is graded of lower quality because of the cellulolytic activity of *Xanthomonas* and other associated microorganisms (Verma, 2000). The bacterium finds its way in to the plant through natural openings or by wounds which can be caused by damage through wind, heavy rainfall, herbicide injury, damage through equipment or insect injury. But stressed plants are not only susceptible to become infected but are prone to express the disease symptoms too. Sometimes, the bacterium enters into the plant by points of injury and then bacterium move throughout the plant in a systemic fashion (Allen, 1991).

Although there are various methods to manage this disease such as chemicals but their use is hazardous to both plants as well as human beings. Although plant allelopathic extracts play an important role in managing diseases while it is slow in action, on the contrary induced resistance developing natural defense use of plants offered as a substitute is ecologically-friendly and conventional way for plant protection. Use of this reduces the choice of chemical use. Induced resistance produced by a series of different elements those debilitated hypersensitive necrotic reaction, pathogenic strains and elicitors of contagious origin are described by (Aglika, 2004). In Pakistan farmer's only use resistant varieties for better production but also resistant varieties production is low as compare to disease tolerant varieties. Induced resistance is normally expressed at the site of infection and systemically near to the initial infection. The purpose of present study was to evaluate different available plant activators against bacterial blight of cotton caused by *X. campestris* pv. *malvacearum* that induce systemic acquired resistance.

MATERIALS AND METHODS

Preparation of land

To conduct the field experiment land was selected in experimental area of Plant Pathology, University of agriculture Faisalabad. The land was leveled with leveler for smooth irrigation and pulverized with cultivators and disc plough to eliminate the weeds. The rainy irrigation was done on 10th of May and after ten days of irrigation soil condition and soil moisture was checked. The land was prepared through cultivator and breaks the soil clods with planker for smooth soil surface.

Experiment layout and plant cultivation

The layout of the experiment was RCBD (Randomized complete block design). The seeds of highly susceptible cotton variety SLH-13 were collected from Cotton research institute (CRI) Sahiwal. The variety was sown in field with row to row distance (R × R) 75cm and plant to plant (P × P) 30cm spacing with the help of hand dibbler.

Preparation, identification and application of Inoculums

The disease inoculums were procured from Central cotton research institute (CCRI) Multan districts of Punjab province of Pakistan. The inoculums were mass culture in plant bacteriology lab in Department of Plant Pathology. A 100 ml conical flask was taken for inoculums culture having 40 ml of nutrient broth at 25°C for 2 days on a shaker at 150 rpm. The bacterial isolate was identified by standard bacteriological tests (Schaad, 1992). The 72 h old culture was diluted in sterile water at the concentration of 10^9CFU/ml in a knapsack sprayer and applied on lower leaves of 28 days old cotton plants (Fallahzadeh and Ahmadzadeh, 2009).

Preparation of plant activators for application

Five plant activators like salicylic acid, K_2HPO_4, KH_2PO_4, citric acid and benzoic acid were sprayed at 1%, 2% and 3% concentrations to induce Systemic Acquired Resistance (SAR) against BLB and 2nd spray was done after 14 days after first application. The treatments were repeated three times and control without any treatment for comparison. The application was done by the mean of knapsack sprayer early in the morning.

Collection of data

Data was recorded at 7 and 14 days of 1st spray application and again after 2nd spray application interval. Fifty leaves were selected randomly from each treated plant row and assessed for bacterial blight symptoms. Disease incidence and severity was noted following the Brinkerhoff (1977) scale and calculated by the following formulas:

$$\text{Disease incidence} = \frac{\text{Disease plants}}{\text{Total plants}} \times 100$$

Assessment of diseases severity of the bacterial blight of cotton caused by *X. compestris* pv. m*alvacearum.*

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves graded} \times \text{maximum grade}} \times 100$$

Statistical Analysis

The statistical test was performed by using SAS statistical software. Means were sorted out by using Fisher's least significant difference (LSD) procedure (Steel *et al.*, 1997).

RESULTS

Effect of plant activators on disease incidence

In current experiment, five plant activators were used like salicylic acid, K_2HPO_4, K_2HPO_4, citric acid and benzoic acid. It was revealed that the first spray of five plant activators with the concentration of 1%, 2%and 3% after 7 and 14 days salicylic acid inhibitthe disease incidence at after 7 days 74%, 76%,75% at 14 days 77%, 77%, 79% respectively followed by citric acid 69%, 71%, 72% after 7 days%, 74%, 77% after 14 days of application. Benzoic acid 62%, 64% and 66% after 7 days 69%, 71%, 69 % after 14 days. K_2HPO_4 60%, 62%, 59% after 7 days 64%, 62%, 64 % after 14 days and KH_2PO_4 52%, 55%,53% after 7 days while 60%, 59%,61% after 14 days as compared to control which shows 51%, 49%, 52%, 50%, 56% and 56 % high disease incidence.

After 15 days of 1st application second spray with same concentration was done disease incidence was recorded after 22 and 28 days. After second application of salicylic acid 76%, 83%, 82% after 22 days and 86%, 84%,88 % disease reduction after 28 days. Citric acid 72%,76%, 77% after 22 days 81%, 81%, 87 % after 28 days, benzoic acid 67%, 71%, 77%, 75%, 74% and 76 %, K_2HPO_4 62%, 65%, 66%, 69%, 66% and 70 % and KH_2PO_4 57%, 61%, 61%, 63%, 62% and 64 % as compared to control 49%, 48%, 49%, 47%, 53% and 53 %.

Table 1. Disease rating scale used to determine the level of resistance or susceptibility of cotton varieties to bacterial blight disease in the field.

Grade	Symptom	Level
0	No macroscopic symptoms	Immune (I)
1-3	Round dry pinhead size lesions developed	Resistant (R)
4-6	Lesions turned to dry angular lesions	Tolerant (T)
7-9	Lesions turned to water soaked spots	Susceptible (S)
10	Spots turned to large angular water soaked lesions on leaf veins	Highly susceptible (HS)

Table 2. Comparison of means of plant activators on disease incidence.

		1st spray		2nd spray	
Treatment	Doses (%)	7 days	14 days	21 days	28 days
Salicylic acid	1	26 fg	24 gh	20 gh	17 fg
	2	25 g	23 gh	18 hi	14 gh
	3	23 g	21 h	16 i	12 h
Citric acid	1	31 de	29 ef	28 e	24 e
	2	29 ef	26 fg	23 fg	19 f
	3	26 fg	23 gh	19 hi	17 fg
Benzoic acid	1	38 c	36 d	33 d	29 d
	2	34 d	31 e	29 e	25 e
	3	29 ef	31 e	26 e	24 e
K2HPO4	1	40 bc	38 cd	38 c	35 c
	2	39 c	36 d	34 d	31 d
	3	38 c	36 d	34 d	30 d
KH2PO4	1	48 a	45 b	43 b	39 b
	2	43 b	40 c	39 c	37 bc
	3	41 bc	39 cd	38 c	36 bc
Control	-	49 a	51 a	51 a	52 a

Means sharing similar letters are statically non-significant (P> 0.05).

Table 3. Comparison of means of plant activators on yield.

Treatment	**Yield**		
	1 %	**2 %**	**3 %**
Salicylic acid	3040c	4160b	4480a
Citric acid	3360d	4040b	3880c
Benzoic acid	2480fg	3040e	2920e
K_2HPO_4	1720j	2600f	2520fg
KH_2PO_4	2040i	2360gh	2200hi
Control	1480k	1720j	1720j
LSD	2.023		

Means sharing similar letters are statically non-significant (P> 0.05).

Effect of plant activators on yield of Cotton

The yield data indicated that salicylic acid significantly increase the yield which was 4480, 4160 and 3040 kg/ha at all concentrations followed by citric acid 3880, 4040 and 3360 kg/ha, benzoic acid 2920, 3040 and 2480 kg/ha, K_2HPO_4 2520, 2600 and 1720 kg/ha and KH_2PO_4 2200, 2360 and 2040 kg/ha.

DISCUSSION

The present study revealed that five plant activators like salicylic acid, K_2HPO_4, K_2HPO_4, citric acid and benzoic acid were used on cotton plants with three concentrations 1, 2 and 3% and *X. compestris* pv. *malvacearum* was inoculated after 24 h. After 14 days of artificial inoculation symptoms in the form of lesions on leaves were observed. Data analysis showed that salicylic acid spray at all concentrations significantly decreased the disease symptoms and increased the yield as compared to control and other plant activators but 2% concentration more affective than other. Citric acid performance was better than benzoic acid, K_2HPO_4, KH_2PO_4 while benzoic acid was better than K_2HPO_4 and KH_2PO_4 and K_2HPO_4 showed better results than KH_2PO_4 in both disease reduction and yield. It may be attributed to the fact that yield directly depends on disease severity.

Salicylic acids induce resistance through acting as a signal molecule and help to trigger plant defense gene expression (Enyedi *et al.,* 1992). Salicylic acids induce resistance in oats against *Drechslera avenae* (Muller and Weltzien, 1990). Salicylic acid enhances both localized acquired resistance (LAC) and systemic acquired resistance (SAR) in plants (Hammerschmidt *et al*., 2001). Benzoic acid also reduces the disease severity of stem rust of rice by acting as abiotic elicitor (Kumar *et al*., 2003).

CONCLUSION

It is confirmed that salicylic acid is the best protectant than other chemicals. Benzoic acid and citric acid are also helpful to manage the disease severity. They also act as bactericide and food preservative but their effect on plant defense mechanism is still unknown. These chemicals may act as protectant against bacterial blight of cotton.

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