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Research article

BIOLOGICAL MANAGEMENT OF BACTERIAL BLIGHT OF COTTON CAUSED BY XANTHOMONAS CAMPESTRIS PV. MALVACEARUM THROUGH PLANT EXTRACTS AND HOMEOPATHIC PRODUCTS

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ABSTRACTS

Bacterial blight is a devastating disease of cotton. Use of plant extracts and homeopathic products are novel in plant disease management. Four plant extracts (*Datura alba*, *Moringa olifera*, *Azadirachta indica* and *Syzgyium cumini*) and three homeopathic products (Aviara, Influenzium and Hepatitus) at 5%, 10% and 15% concentrations against colony of *Xanthomonas campestris* pv. *malvacearum* by inhibition zone technique. *Datura alba* was the most significant to inhibit bacterial colony (2.1 cm) at 15% dose after 72 hours. Influenzium was the most significant homeopathic product retarding bacterial growth (1.70 cm) at 15% concentration. In green house and in field conditions, treatments efficacies were assessed on four cotton varieties (FH-941, FH-942, MNH-886 and FH-114). Significant decrease in disease incidence was observed by *Datura alba* on MNH-886 (39.75%) as compared to Influenzium (43.25%) and negative control (48.50%).Three consecutive sprays were done at 7 days interval. Disease incidence significantly decreased by *Datura alba* after 3rd spray as compared to Influenzium and control (Negative) but not from Streptomycin sulphate (positive control).

Keywords: *Xanthomonas campestris* pv. *malvacearum*, Influenzium, *Datura alba*

INTRODUCTION

Cotton is a fiber cash crop extensively sown throughout the world. It belongs to family "Malvaceae" and genus "Gossypium" having 35 cotton species. Cotton is broadly grown in tropical and subtropical regions. In world, cotton is cultivated under the area of 35.7 million hectares with 123.3 million bales yield [1]. Diseases and pests significantly reduce cotton yield. Bacterial blight of cotton is a serious threat to cotton, average losses by disease vary from region to region. In Pakistan 50% yield losses have been estimated under optimal conditions [2]. Bacterial blight of cotton is incited by *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye. Disease primarily spreads through infected seeds. In seedling stage, extensive circular spots form on cotyledons leaves resulting into failure of seedling development due to reduced photosynthetic area. Water soaked spots appear on leaf surface which latterly turns in brown and corky as disease progresses. In severe conditions, stem girdles by angular spots and easily breaks us at low wind speed. Use of homeopathic is novel in plant disease management but did not gain much interest by scientists. These are unique in its action not only inhibiting pathogen growth but also have tonic effect on plant health. Homeopathics are cheap and eco-friendly, used in a very low doses [3]. Plants have natural antimicrobial substances having toxic effect against pathogens. However, a little is known what pathways are involved to suppress the pathogen growth and development. Plant extracts are easy to synthesize and have broad spectrum use.

Study was proposed to evaluate the effect of different homeopathics and plant extracts at various doses against colony growth of *Xanthomonas campestris* pv. *malvacearum* in lab conditions. Significant doses from each treatment showing maximum inhibition zones were selected for green house and field disease management.

MATERIALS & METHODS

Cotton leaves having typical disease symptoms were washed with tap water. After drying disease tissues were excised into small pieces and surface sterilized by 0.1% $HgCl_2$ and rinsed twice with distilled water. After drying on blotter paper, one gram diseased tissues were macerated with one milliliter of water by pestle and mortar. Bacterium was isolated from infected tissues by dilution plate technique on nutrient glucose agar (nutrient glucose=28g & water=1L) medium. Plates were wrapped and incubated for 48 hours at 32 ^{c}ob Bacterial colonies were purified by streaking plate method and identified morphologically.

Leaves of four plants (*Datura alba, Moringa olifera, Azadirachta indica* and *Syzygium cumini*) were taken and washed thoroughly with tap water and air dried. Twenty five gram leaves of each plant were measured and grinded with 75 ml of distilled water. Supernatant was separated finely by glass pipette and considered as standard. Three dilutions were made @ 5%, 10% and 15% of each plant extract by adding requisite amount of sterile water. Inhibition zone technique was used to assess the efficacy of each extract at different doses against colony growth of *Xanthomonas campestris* pv. *malvacearum* in lab condition. Streptomycin sulphate served as positive control. In negative control, water was poured. Plates were wrapped and incubated at 32 ^{o}C for 48 hours. Treatments were arranged in Completely Randomized Design with three replications. Data was recorded by measuring inhibition zone diameter after 24, 48 and 72 hours of treatment by an ordinary ruler in centimeter scale.

Three homeopathic products (Aviara 30CH, Influenzium 30CH and Hepatitus 30CH) were purchased from a drug store and evaluated against colony growth of *Xanthomonas campestris* pv. *malvacearum*. Three doses 5%, 10% and 15% were made by adding requisite sterile water. Efficacy of each treatment (Aviara 30CH, Influenzium 30CH and Hepatitus 30CH) at 5%, 10% and 15% was evaluated by inhibition zone technique [4].

In green house and in field conditions, one treatment from each (homeopathic products and plant extracts) with significant efficacy in *in-vitro* experiments was selected for field trials. Acid-delinted seeds of four cotton varieties (FH-941, FH-942, MNH-886 and FH-114) were sown in experimental area of Plant Pathology, University of Agriculture, Faisalabad. Homeopathic product and plant extract were sprayed on foliar part of cotton plants. In control distilled water was applied. The natural inoculums was relied upon for infection. Data was recorded after 7 days of treatment. Trial was conducted in Randomized Complete Block Design (RCBD) with three replications.

Statistical analysis

Analysis of Variance (ANOVA) was performed to statistically analyze experimental data, treatments means were compared by Fisher's Least Significance Difference (LSD) test [5]. Statistical analysis was performed by SAS (9.3) software[6] and graphical representation was executed through "Microsoft Excel".

RESULTS

Yellowish colonies appeared after 48 hours. Purified colonies were identified as *Xanthomonas campestris* pv. *malvacearum* on the basis of morphological and biochemical characters [7]. When inoculated on greenhouse grown cotton plants, these bacterial colonies produced characteristic bacterial blight symptoms.

In vitro evaluation of plant extracts

Significant increase in inhibition zone area was noticed with respect to time of treatment (24, 48 and 72hours). Significant results were observed among four plant extracts. Three concentrations (5%, 10% and 15%) significantly influenced inhibition zone diameter with respect to time. Treatment efficacy increased against bacterial colony growth with respect to time interval. Significant interaction was seen among time, treatments and doses (Table I). After 24 hours of treatment, significant results were noticed by *Datura alba* (0.1 cm) while no effect was noticed by rest of plant extracts at 5% concentration. At 10% dose, *Datura alba* was the most significant (0.20 cm) to inhibit bacterial colonies followed by *Syzgium cumini* (0.10 cm). Streptomycin sulphate (positive control) made 0.13 cm inhibition zone. Similar trend was seen at 15% concentration after 24 hours of treatment except positive control (Streptomycin sulphate). In control no zone was observed (Fig I). After 48 hours, significant increase in inhibition zones was noticed by all treatments as compared to after 24 hours. Significant increasing trend was noticed with increase of concentration used. *Datura alba* was most significant at 5%, 10% and 15% concentrations as compared to other plant extracts but was less significant as compared to streptomycin sulphate (positive control).Similar trend was noted at 10% and 15% doses (Fig I). After 72 hours, inhibition zones significantly increased as compared to 24 hours and 48 hours of periods. At 5% and 10%, *Datura alba* was the most significant as compared to other plant extracts and positive control as well. No significant difference was observed between *Datura alba* and streptomycine sulphate. *Moringa oleifera* was the least significant to inhibit colony growth of *Xanthomonas campestris* pv. *malvacearum.* In control (negative), no zones formed after 24 hours, 48hours and 72 hours (Fig 1).

In vitro evaluation of homeo products

Significant increase in inhibitions zones area was observed by increase of dose and passing of time (hours) of evaluation periods. Significant interaction was found among time (hours), doses and treatments (including positive and negative control) (Table II). After 24 hours, no zones were recorded by aviaria and Hepatitus at 5% concentration. No significant difference was observed between Influenzium and Streptomycin sulphate (positive control). At 10% dose, Influenzium was the most significant (0.17 cm) to inhibit colony growth of *Xanthomonas campestris* pv. *malvacearum* as compared to others (including positive control). Influenzium significantly reduced bacterial colony diameter at 15% than other homeo products but was less significant to Streptomycin sulphate (Fig II). After 48 hours, significant difference in increase of inhibition zone was recorded. Among homeo products, Influenzium was most significant in reducing bacterial colony diameter at 5, 10 and 15% followed by aviara. Inhibition zone diameter significantly increased after 72 hours of treatment, similar trend was observed as was noticed after 48 hours. In negative control, no zone was recorded irrespective to time (24 hours, 48hours and 72 hours). Influenzium remained the most significant (1.7 cm) and aviara was at second (1.2 cm) to inhibiting colony growth of *Xanthomonas campestris* pv. *malvacearum* in lab conditions (Fig II). Homeopathic products are prepared by repeated dilutions of active substance in alcohol or in distilled water. Apparently, it is not known how these products inhibit microbial growth. Further study is needed to explore the mechanisms and pathways involved for inhibition or damaging of bacterial cells.

Management of disease in green house and field conditions through plant extract and homeo product

Significant difference was noted among treatments applied to manage disease incidence. No significant difference in disease incidence response was observed by four cotton varieties (FH-941, FH-942, MNH-886 and FH-114). Significant interaction was found between varieties and treatments (Table III). *Datura alba* significantly reduced disease incidence on MNH-886 as compared to other cotton varieties (FH-941, FH-942 and FH-114).Streptomycin sulphate (positive control) was the most significant as compared to other treatments, reduced significantly disease incidence on FH-114 cotton variety. Influenzium was least significant as compared to Influenzium and Streptomycin sulphate (positive control) except negative control (untreated) (Fig III).
In field conditions, significant difference was observed among three consecutive sprays and treatments. Significant decrease in disease incidence by different treatments at three different sprays. Among four cotton varieties (FH-941, FH-942, MNH-886 and FH-114), no significant difference in disease response was observed. Non-significant interaction was seen among sprays, varieties and treatments (Table IV). After first spray, Streptomycin sulphate (positive control) was the most significant in disease control in contrast to Influenzium (homeo product) and *Datura alba*. *Datura alba* was more significant than Influenzium (homeo product) and negative control. Similar trend was observed after 2nd and 3rd spray (Fig IV). From this experiment, significantly disease managed as compared to control (negative control) treatment by Influenzium and *Datura alba* was recorded.

DISCUSSION

Hyoscine (alkaloid) has been abundantly found as a toxic compound in *Datura alba* leaves, disrupting cell wall's integrity by making complexes with cholesterols and ultimately interrupts with phospholipids motion. In results, tension produces and cell wall ruptures[8]. Generally in eukaryotic cell, it binds with to muscarinic acetylcholine receptors and acts as competitive antagonists. Khan, Rashid and Chohan [9], evaluated leaves extracts of *Datura alba*, neem seed oil, neem seed bitter and commercial neem based product "Nimbokil (40 EC)" at 1, 2 and 3% concentration against *Xanthomonas campestris* pv. *malvacearum* colony growth. No zone was observed at 1% by any treatment. *Datura alba* at 3% was the most significant to inhibit colony growth after 72 hours of treatment. Sajid, Rashid, Ehetisham-ul-Haq, Javed, Jamil, Mudassir, Farooq, Ahmad, Latif, Chohan, Ahmad and Kamran [10], assessed efficacy of three plant extracts (*Catullus colocynths, Nicotiana tobaccum and Curcuma lunga*) at 5%, 10% and 15% against *Xanthomonas campestris* pv. *malvacearum. Nicotiana tobaccum* was most significant at 10% after 72 hours against bacterial blight of cotton pathogen. Seventeen plants extracts were screened by Ravikumar, Selvan and Gracelin [11]against fungal and bacterial pathogens. Out of 17 plants, 6 were found effective. *Datura metal* was the most effective for *Pithier restricted* fungal species (19 mm) and for Gram negative bacteria (13mm) than other plants extracts used.

Khanna and Chandra [12], significantly controlled tomato rot caused by *Fusarium roseum* using two homeo products i.e. Kali iodatum at 149CH and Thuya occidentalis at 87CH in pre and post-harvest conditions. Later on effect of various homeopathics was assessed against spore germination and respiration of fungi e.g., *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium roseum*and *Gloeosporium psidii* [13, 14]. Sinha and Singh [15]evaluated different homeo drugs against various fungal species, 100% growth inhibition of *Aspergillus parasiticus* was recorded by Sulphur (200CH). Silicea terrea and Dulcamara reduced 50% fungal growth and 90% toxins production, phosphorus imposed little effect on fungal growth inhibit but reduced (30%) significantly aflatoxins production. Aiming to trigger resistance against bacterial spot of tomato caused by *Xanthomonas campestris*, significant reduction in disease severity was noticed at 6CH and 24CH dynamizations [16].

Bacterial blight of cotton spreads primarily through contaminated seeds. Bacterium (*Xanthomonas campestris* pv. m*alvacearum*) moves from seed to cotyledons leaves. Secondary infection occurs when pathogen enters to plant through leaves stomata or wounds by rubbing of infected leaves or due to contaminated irrigation water. The only possibility remains to control the disease is through systemic chemotherapeutants. Yet not known which mechanism or pathways involved in for controlling the pathogen (*Xanthomonas campestris* pv. *malvacearum*) by Influenzium (homeo product) certain hypothesis emerges like, whether homeo products trigger defense

mechanism of plant or it directly effects pathogen's biotic pathways. These needs biochemical approaches to explore the answers. As discussed above, *Datura alba* leaves mainly have alkaloid (hyscoine) which disrupts bacterial cell wall. However, nothing is known about its persistency and pathway in plant system against bacterial blight of cotton pathogen.

Raghavendra, Lokesh and Prakash [17] soaked seeds and made three foliar sprayings of Drava (seaweed) to manage bacterial blight of cotton at 10 days consecutive intervals. Significant results were seen in a reduction of blight incidence. Toledo, STANGARLIN and BONATO [18] assessed two homeo products Sulphur and Ferrum sulphuricum to control early blight on tomato plants. Sulphur at 12 and 30CH significantly minimized disease severity after ten days of inoculation with *Alternaria solani*. Ferrum sulphuricum at 12 and 60CH reduced the severity as well after ten but not induced systemic resistance. Rolim, Tofoli, Domingues and Rossi [19] applied Staphysagria 30CH to tomato plants, in greenhouse, reduced the severity of early blight. Phosphorus30CH and 60CH significantly reduced of *A. solani* infection indicating that the use of homeopathy as technically feasible for plant disease control.

CONCLUSION

From this experiment it is concluded that plant extracts and homeopathic products had significant result against pathogen. *In vitro* the *Datura alba* and Influenzium from plant extracts and homeopathic products respectively showed the maximum result against the bacterial colony growth at 15% concentration among the other treatments. And in greenhouse and field management of disease was also controlled by *Datura alba* followed by the influenzium and control (un-treated). It was also concluded that these are least expensive and not irritating the ecosystem, so, they are preferred on the use of other synthetic chemicals (pesticides).

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Table I: Analysis of Variance (ANOVA) for the effect of different plant extracts on bacterial blight of cotton pathogen

Source	DF	SS	MS	F	P
Hours	2	12.7298	6.36491	667.00	0.0000**
Doses	2	8.3410	4.17052	437.04	0.0000**
Treatments	5	14.2082	2.84164	297.78	0.0000**
Hours x Doses	4	1.8118	0.45296	47.47	0.0000**
Hours x Treatments	10	5.5572	0.55572	58.24	0.0000**
Doses x Treatments	10	4.6816	0.46816	49.06	0.0000**
Hours x Doses x Treatments	20	1.2009	0.06005	6.29	0.0000**
Error	108	1.0306	0.00954		
Total	161	49.5612			

** = Highly significant

α = 0.05

Table II: Analysis of Variance (ANOVA) for the effect of different homeopathic products on bacterial blight of cotton pathogen

Source	DF	SS	MS	F	F
Hours	2	7.9523	3.97613	616.99	0.0000**
Doses	2	6.6440	3.32202	515.49	0.0000**
Treatments	4	10.3540	2.58849	401.66	0.0000**
Hours x Doses	4	1.1724	0.29310	45.48	0.0000**
Hours x Treatments	8	3.2474	0.40592	62.99	0.0000**
Doses x Treatments	8	3.7178	0.46473	72.11	0.0000**
Hours x Doses x Treatments	16	0.8902	0.05564	8.63	0.0000**
Error	90	0.5800	0.00644		
Total	134	34.5580			

** = Highly significant

α = 0.05

Table III: Analysis of Variance for the response of plant extract and homeopathic product against bacterial blight of cotton in greenhouse condition.

Sources	DF	SS	MS	F	P
Treatments	3	642.125	214.042	109.88	0.0000**
Varieties	3	4.375	1.458	0.75	0.5285^{NS}
Treatments x varieties	9	39.750	4.417	2.27	0.0331*
Error	48	93.500	1.94b		
Total	**63**	**779.750**			

** = Highly significant * = significant

NS= Non-significant α = 0.05

Table IV: Analysis of Variance for the response of plant extract and homeopathic products against bacterial blight of cotton in field condition.

Sources	DF	SS	MS	F	P
Replications	2	9.76	4.88		
Spray	2	184.35	92.17	16.96	0.0000**
Variety	3	25.81	8.60	1.58	0.1988^{NS}
Treatment	3	6027.81	2009.27	369.68	0.0000**
Spray x Variety	6	1.65	0.28	0.05	0.9994^{NS}
Spray x treatment	6	170.99	28.50	5.24	0.0001*
Variety x Treatment	9	61.47	6.83	1.26	0.2709^{NS}
Spray x variety x Treatment	18	6.57	0.36	0.07	1.0000^{NS}
Error	94	510.90	5.44		
Total	**143**	**6999.31**			

** = Highly significant * = significant

NS= Non-significant $\alpha = 0.05$

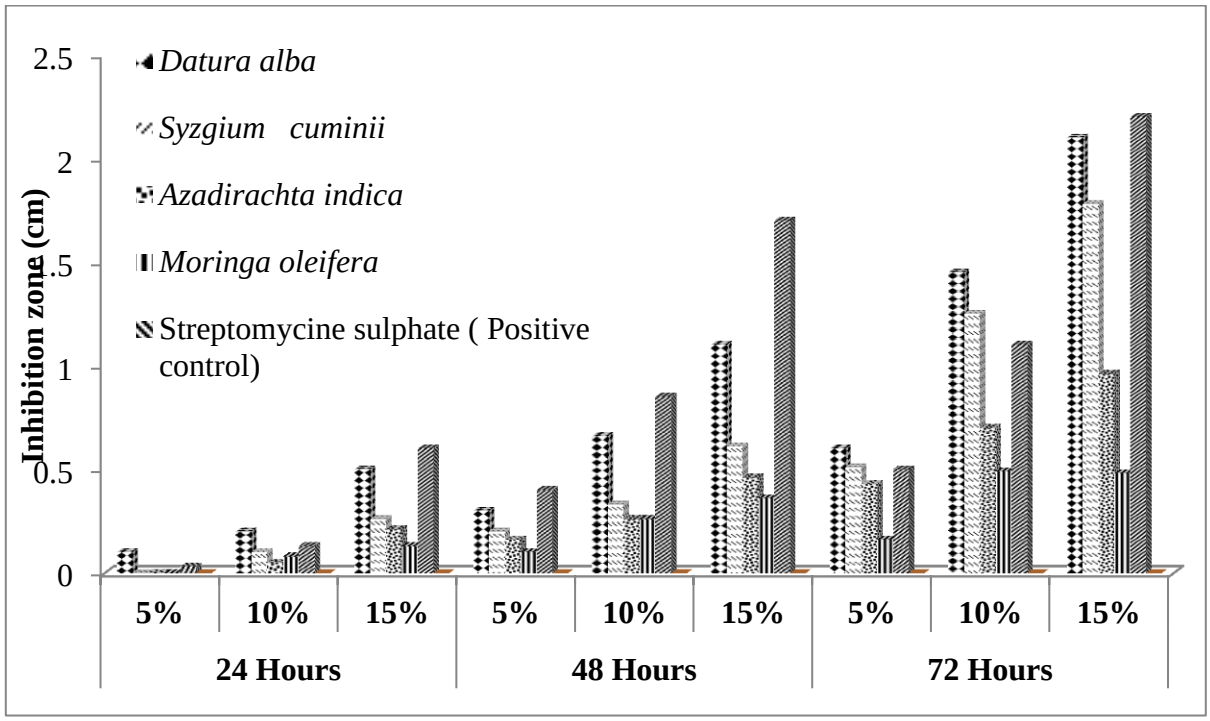

Figure I: Representation of plant extracts efficacies at different doses regarding to time interval against bacterial colony growth

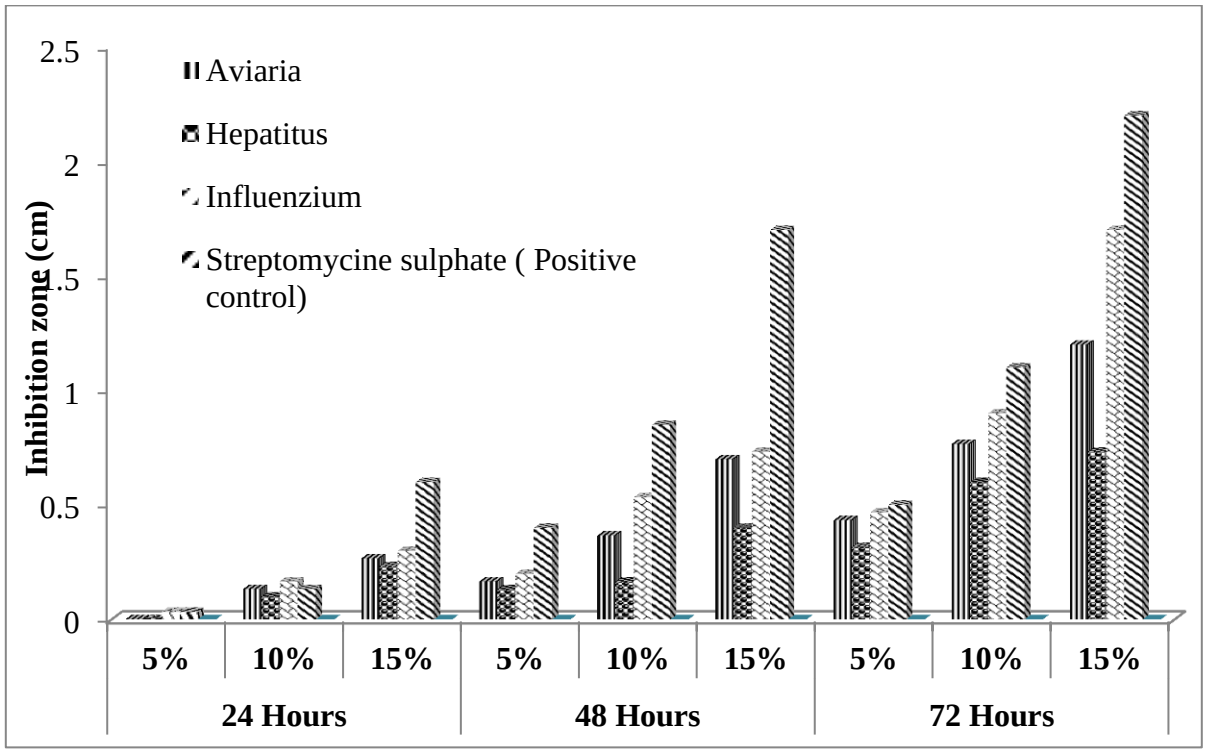

Figure II: Representation of Home products efficacies at different doses regarding to time interval against bacterial colony growth

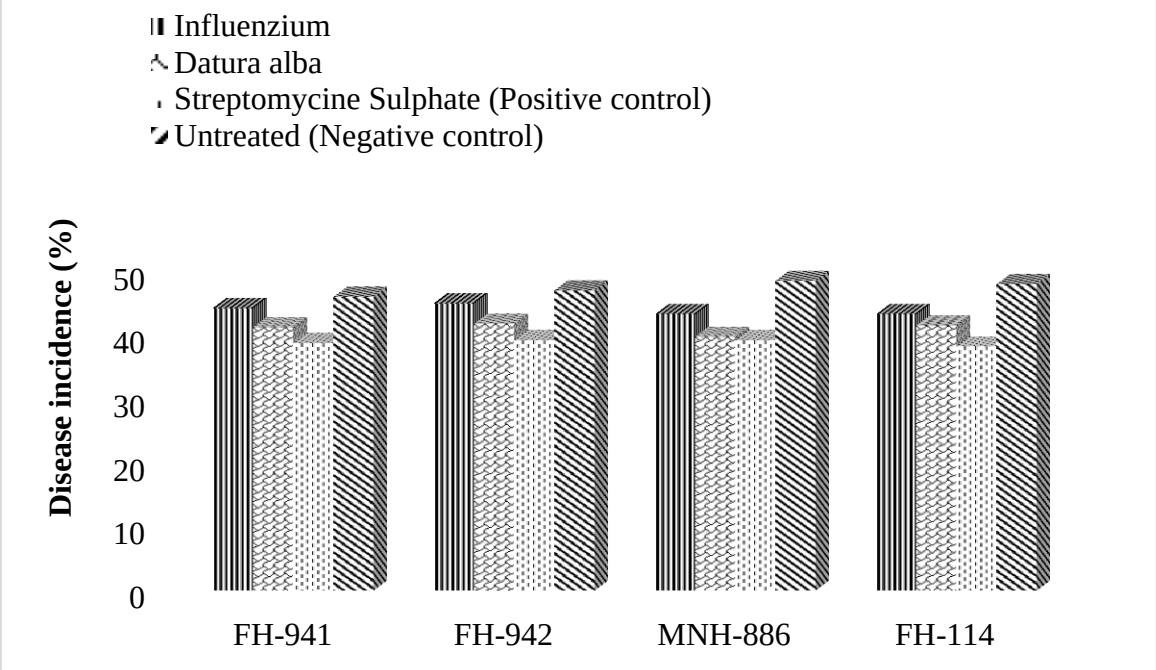

Figure III: Representation of plant extract and homeo product efficacies against bacterial blight of cotton in green house conditions

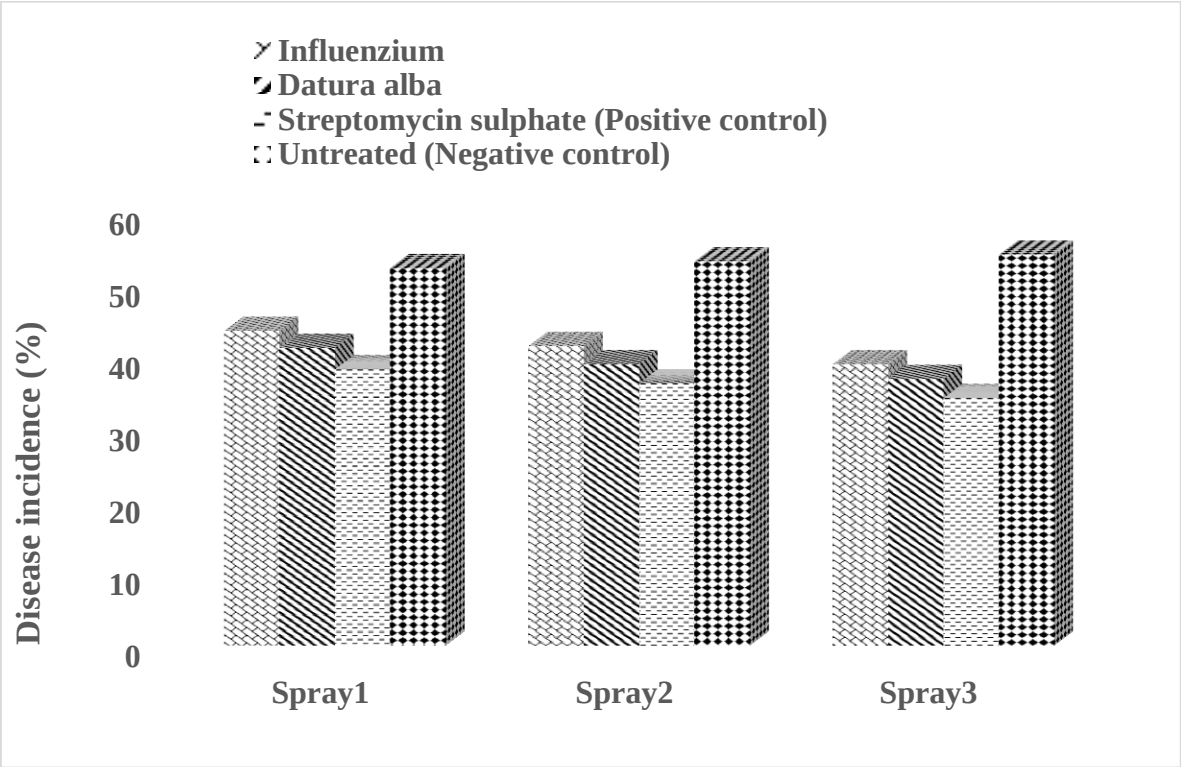

Figure IV: Representation of plant extract and homeo product efficacies against bacterial blight of cotton in field condition.