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Evaluation of effective bio-agents and botanicals against *X. a.* pv. *punicae* under field condition

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Abstract
A field study was conducted to evaluate the effectiveness of bio-agents and botanical extracts against *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight in pomegranate. The study involved multiple treatments, including bacterial bio-agents (Isolate SM-1A, VK-6B, BK-6, KK-9A) and botanical extracts (garlic, kokum, prosopis), a standard check (Streptocycline + COC), and an untreated control. Treatments were applied five times at fortnightly intervals and various parameters were assessed. Garlic extract exhibited significant disease reduction, with notable percentage reductions in lesions per leaf (77.80%), leaf infection (79.61%), leaf area infected (81.08%), twig infection (71.43%), twig lesion size (75.96%), lesions per fruit (73.63%), and fruit infection (72.47%). Standard check treatment demonstrated the highest potency in disease reduction across multiple parameters. Kokum extract and VK-6B bio-agent isolate also showed promise in disease management. The study underscores the potential of garlic extract, VK-6B bio-agent, and kokum extract for effective and eco-friendly control of pomegranate bacterial blight. Further research is warranted to explore active compounds and refine disease management strategies.

Keywords: Pomegranate bacterial blight, *Xanthomonas axonopodis* pv. *punicae*, bio-agents, botanicals, disease management

Introduction
Pomegranate (*Punica granatum* L.) is an important fruit crop both in India and around the world. It belongs to the Punicaceae family (Malhotra *et al*., 1983) [12] and originates from Iran. However, it is extensively grown in countries such as Spain, Morocco, Egypt, Iran, Afghanistan, and other Mediterranean areas. In India, the total pomegranate cultivation area is 1.43 lakh hectares, with Maharashtra (99,140 ha) followed by Karnataka (19,040 ha) leading the states. Moreover, the total production in India is 1773.66 metric tons, with Maharashtra (13,13,370 MT) and Karnataka (2,04,640 MT) being the most contributing states. Interestingly, Tamil Nadu claims the lead in productivity (34.43 Mt/ha), followed by Telangana, Maharashtra, Andhra Pradesh, and Karnataka. Despite ranking second in both area and production, Karnataka holds the fifth spot in terms of productivity. This lower productivity is attributed to the incidence of pests and diseases, including bacterial blight, wilt, anthracnose, leaf and fruit spots, and nematodes. Among these, the bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* plays a significant role in the reduction of production and productivity.

The emergence of bacterial blight disease in 2001 was initially documented by Manjula and Khan (2002) [13] in specific parts of Karnataka and Maharashtra, marking the beginning of its rapid spread across pomegranate-growing regions in India. However, despite its profound impact, studies on bacterial blight remain relatively sparse within the Indian context. A noteworthy contribution by Benagi *et al*. (2009) [3] assessed disease severity during "mrig bahar," revealing a range between 0.67% and 94.80% across diverse pomegranate-growing zones. The detailed symptomatic expression of bacterial blight on pomegranates at different stages was recorded by Ramesh Ippikoppa *et al*. (2017) [20]. Leaves bear the initial brunt, displaying minute water-soaked lesions that evolve into brown to dark brown spots, often encircled by a diffused water-soaked margin or a yellow halo. Over time, these spots converge on a single leaf, giving rise to irregular, expanding lesions. Afflicted leaves eventually distort, turn yellow, and ultimately defoliate. On main stems, twigs, and branches, elongated bluish-black lesions emerge, which later transform into rough, cankerous, dark brown patches. The bark's gradual deterioration manifests through cracking, drying, and eventual decay.

Fruits, too, succumb to the disease, with initial appearances of small, diffuse oily lesions that progressively turn brown and become encircled by diffused water-soaked zones. The lesions, once circular, expand into irregular, dark brown patches. The effect on the fruit rind is conspicuous, resulting in cracks that adopt L, Y, or star shapes. As the disease advances, these lesions incite longitudinal splits in affected fruits, thus exposing arils.

To manage this disease under field conditions, many bactericides are available in the market and are effective. However, a major concern is the presence of chemical residue on fruits. Chemical use not only fails to provide a comprehensive solution for disease management but also contributes to pollution and ecological hazards. Additionally, pomegranate exports to European and American markets are subject to strict grading standards, particularly regarding residual toxicity levels for pesticides, discouraging the use of antibiotics/bactericides. Consequently, there is a pressing need to develop alternative and eco-friendly measures for disease control.

Biological control of plant pathogens through antagonistic microorganisms is a potential non-chemical approach (Harman, 1991) [7] and is recognized as a cost-effective and environmentally friendly method for managing crop diseases (Cook and Baker, 1983) [4]. The members of the Pseudomonas and Bacillus genera have long been acknowledged for their potential in reducing bacterial plant diseases and have gained importance as potential antagonistic microorganisms (Pant and Mukhopadhyay, 2001) [17]. Considering this, a biological approach to manage bacterial blight of pomegranate involves collecting native isolates of bio-agents from different rhizosphere soils and evaluating their antagonistic effect against *Xanthomonas axonopodis* pv. *punicae*. Certain antagonistic bio-agents aggressively colonize the root zone and promote plant growth. Primarily, Pseudomonas fluorescens has been identified as an organism with the ability for plant growth promotion and effective disease management properties (Mazzola *et al*., 1992) [14]. Similarly, bio-control agents produce secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes, and phytohormones, hindering the growth and multiplication of plant pathogenic bacteria (Nagarajkumar *et al*., 2004) [16]. Another non-chemical method involves botanicals, which are of natural origin, biodegradable, and do not leave toxic residues or byproducts in the environment. Moreover, botanical pesticides are cost-effective compared to chemical pesticides.

Therefore, bacterial bio-agents and botanicals are considered ideal disease management strategies to be employed for bacterial blight of pomegranate. Hence, in the previous studies, we collected, evaluated, and identified 13 effective bio-agents ((SM-1A, VK-6B, BK-6, KK-9A, VK-10C, KK-3A, BK-5, BK-3, CK-13A, BK-8, SM-2A, BK-7, and BK-1L)) against *Xanthomonas axonopodis* pv. *punicae* (Ramesh Ippikoppa *et al*., 2023a) [18] and further characterized them morphologically and biochemically (Ramesh Ippikoppa *et al*., 2023b) [19]. Thus, the current study was undertaken to evaluate the performance of identified effective bio-agents along with different botanicals against *Xanthomonas axonopodis* pv. *punicae* under field conditions.

Material and Methods

An experiment was conducted during 2015 (May to July) at a farmer's field in Sokanadagi village, Bagalkot taluk, Bagalkot district, Karnataka, India, using the cultivar Kesar. The experimental design employed was a Randomized Block Design (RBD) consisting of nine treatments with three replications, and each replication contained three plants. The treatment details are as follows, T_1- Isolate SM- 1 A @ 10^8 cfu /ml ; T_2- Isolate VK-6B @ 10^8 cfu /ml ; T_3- Isolate BK- 6 @ 10^8 cfu /ml; T_4- Isolate KK- 9A @ 10^8 cfu /ml ; T_5- Kokum extract (*Garcinia indica*) @ 1:5; T_6-Prosopis extract (*Prosopis juliflora*) @ 1:5 ; T_7- Garlic extract (*Allium sativum*) @ 1:5; T_8- Standard check (Streptocycline 0.5 g/L (500 ppm) + COC 3 g/L (0.3%); T_9- Untreated control (water spray) and Each treatment received five sprays at fortnightly intervals for application.

Multiplication of Bioagents (Vijayan *et al*., 2013) [24]

Liquid mother cultures of the four isolates were prepared in separate test tubes. The sterilized conical flasks containing prepared nutrient agar broth were gently opened, and 1 ml of each liquid mother culture was carefully poured into the liquid medium. The flasks were closed with cotton plugs and incubated in an orbital shaker at 30 °C and 200 rpm for 72 hours. The resulting bacterial culture was diluted with water (100 ml to 10 liters) to achieve a population of 108 cfu/ml for field application.

Extraction of Plant Extracts

1 kg of each of the three different plants (kokum, prosopis, and garlic) was washed, ground in a 1:1 ratio in a mixy jar, and left overnight for metabolite release. The mixture was then filtered using muslin cloth, and 5 liters of water was added to the solution to achieve a 1:5 dilution. This diluted solution was sprayed onto the pomegranate plants in the field.

Further, following observations were recorded, Number of lesions per leaf (Counted in 27 leaves per plant i.e. 9 upper leaves, 9 middle leaves, and 9 lower leaves, and the average value was recorded); Percentage of leaves infected (%) (Recorded based on the number of infected leaves out of a hundred leaves/plant); Percentage of leaf area infected (%) (Based on the percentage of leaf area covered by the disease in 27 leaves/plant); Number of twigs infected (Counted the total number of twigs infected by bacterial blight per plant, and the average value was recorded); Size of lesions on twigs (cm) (Measured the length of lesions on 3 infected twigs using a scale, and the average value was recorded); Number of lesions per fruit (Counted in 9 fruits per plant, and the average value was recorded); Percentage of fruit infection (Recorded based on the number of infected fruits/plant).

Results

A field experiment was conducted from May to July 2015 in a farmer's field at Sokanadagi village in Bagalkot taluk to assess the effectiveness of various isolates of bacterial bio-agents and botanicals for the control of bacterial blight in pomegranate (Fig. 1). The results obtained from this study are outlined in Tables 1-7 and depicted in Figures 2-4.

Number of Lesions per Leaf

The data regarding the number of lesions per leaf, influenced by the various treatments, revealed significant differences between the treatments (Table 1). The highest percentage reduction in the number of lesions per leaf was observed in the standard check treatment, Streptocycline @ 500 ppm +

COC @ 0.3%, resulting in a reduction of 80.00%. This reduction was comparable to that achieved using garlic extract @ 1:5 (77.80%). Both of these treatments outperformed all other interventions. The treatment involving isolate VK-6B @ 108 cfu/ml (61.90%) showed the next best reduction, which was on par with the reduction observed with kokum extract @ 1:5, resulting in a reduction of 58.33%.

In the standard check treatment, the number of lesions per leaf was 5.0 before the initial spray, and it decreased to 4.67, 3.67, 2.67, 1.33, and 1.00 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. Similarly, for the garlic extract treatment, the number of lesions per leaf decreased from 3.00 before the spray to 2.67, 2.33, 2.00, 1.33, and 0.67 after each successive spray. Conversely, in the treatment involving isolate VK-6B @ 108 cfu/ml, the number of lesions per leaf was 7.00 before the initial spray and reduced to 6.67, 6.00, 4.00, 3.33, and 2.67 after subsequent sprays. Similarly, for plants treated with Kokum extract @ 1:5, the number of lesions per leaf decreased from 4.00 before the initial spray to 3.67, 3.33, 3.00, 2.67, and 1.67 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. In the untreated control group, the number of lesions per leaf was recorded as 4.67 before the initial spray and increased to 5.00, 5.33, 5.67, 6.00, and 6.67 at each subsequent stage.

Per cent leaves infected

The most substantial reduction in the percentage of leaves infected was observed in the standard check treatment (Streptocycline @ 500 ppm + COC @ 0.3%), with a significant reduction of 83.52%. Following this, the botanical garlic extract @ 1:5 showed a reduction of 79.61%. The bio-agent isolate VK-6B @ 108 cfu/ml demonstrated the next best reduction of 74.26%, while plants treated with kokum extract @ 1:5 exhibited a reduction of 60.23% (Table 2 and Fig. 2). The lowest reduction percentage was observed in isolate SM-1A @ 108 cfu/ml (21.10%), followed by isolate BK-6 @ 108 cfu/ml, which showed a reduction of 38.13%.

In the standard check treatment, the percentage of leaves infected was 40.45% before the initial spray, but decreased to 39.33%, 31.67%, 22.33%, 13.67%, and 6.67% after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. Similarly, for the garlic extract treatment, the percentage of leaves infected decreased from 39.78% before the spray to 37.33%, 30.67%, 25.67%, 14.00%, and 8.11% after each successive spray.

For the treatment involving isolate VK-6B @ 108 cfu/ml, the percentage of leaves infected was 45.33% before the initial spray and reduced to 41.67%, 39.33%, 26.00%, 19.67%, and 11.67% after subsequent sprays. Likewise, in the case of plants treated with kokum extract @ 1:5, the percentage of leaves infected decreased from 29.33% before the initial spray to 27.67%, 25.00%, 20.00%, 16.00%, and 11.67% after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. The percentage of leaves infected gradually increased in the untreated control group, which recorded 39.11% before the initial spray and increased to 43.67%, 44.00%, 48.33%, 51.00%, and 57.00% at each subsequent stage.

Per cent leaf area infected

The lowest reduction percentage was observed in plants treated with isolate SM-1A @ 108 cfu/ml (28.57%), followed by isolate KK-9A @ 108 cfu/ml, which recorded a reduction of 31.48% (Table 3). Conversely, the plants treated with the standard check exhibited the highest reduction in percentage (87.64%), significantly differing from the others. Following this, garlic extract @ 1:5 demonstrated a reduction of 81.08%. The treatment involving bio-agent isolate VK-6B @ 108 cfu/ml showed a reduction of 73.81%, and the next best treatment was kokum extract @ 1:5, which recorded a reduction of 70.59%. In the standard check treatment, the percentage of leaf area infected was 29.67% before the initial spray and decreased to 25.00%, 20.67%, 12.67%, 8.00%, and 3.67% after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. Similarly, for the garlic extract treatment, the percentage of leaf area infected was 24.67% before the spray and decreased to 22.00%, 19.00%, 14.33%, 9.33%, and 4.67% after each successive spray.

In the treatment involving isolate VK-6B @ 108 cfu/ml, the percentage of leaf area infected was 28.00% before the initial spray and decreased to 26.33%, 24.67%, 18.00%, 12.67%, and 7.33% after subsequent sprays. Similarly, for plants treated with kokum extract @ 1:5, the percentage of leaf area infected was 34.00% before the initial spray, which decreased to 32.67%, 27.33%, 20.67%, 14.67%, and 10.00% after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. In the untreated control group, the percentage of leaf area infected was 29.00% before the initial spray and gradually increased to 31.67%, 36.33%, 38.00%, 41.67%, and 48.67% after each successive spray.

Number of Infected Twigs

The highest percentage reduction in the number of infected twigs was observed in the standard check treatment (Streptocycline @ 500 ppm + COC @ 0.3%), resulting in a significant reduction of 76.92%. This treatment exhibited a significant difference from all other treatments. Following this, the garlic extract @ 1:5 treatment demonstrated a reduction of 71.43%. The next most effective treatment was the bio-agent VK-6B isolate @ 108 cfu/ml, which displayed a reduction of 66.67%, followed by kokum extract @ 1:5 with a recorded reduction of 59.09% (Table 4 and Fig. 3).

The initial number of infected twigs was 4.33 before the commencement of spraying, and it decreased to 4.00, 3.33, 2.33, 1.67, and 1.00 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively, in the case of the standard check treatment. Similarly, for the garlic extract treatment, the number of infected twigs decreased from 2.33 before the spray to 2.00, 2.00, 1.67, 1.00, and 0.67 after each successive spray.

For the isolate VK-6B @ 108 cfu/ml treatment, the number of infected twigs was 5.00 before the initial spray and reduced to 4.67, 4.00, 3.33, 2.67, and 1.67 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. In the case of plants treated with Kokum extract @ 1:5, the number of infected twigs decreased from 7.33 before the initial spray to 7.00, 6.67, 5.00, 4.00, and 3.00 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. However, in the untreated control group, the number of infected twigs was 5.00 before the initial spray and increased to 5.33, 5.67, 6.33, 6.67, and 7.33 after each subsequent spray.

Size of lesions on twigs

The highest percentage reduction in the size of lesions on twigs was observed in plants treated with the standard check (Streptocycline @ 500 ppm + COC @ 0.3%), resulting in a significant reduction of 78.95%. This treatment outperformed all other interventions. Following this, the garlic extract @ 1:5 treatment demonstrated a reduction of 75.96%. The next most

effective treatments were the bio-agent VK-6B isolate @ 108 cfu/ml and kokum extract @ 1:5, which recorded reductions of 63.49% and 50.50% respectively (Table 5 and Fig. 3). In the standard check treatment, the size of lesions on twigs was 3.17 before the initial spray, and it decreased to 3.00, 2.50, 1.50, 1.00, and 0.67 after the 1st, 2nd, 3rd, 4th, and 5th sprays, respectively. Similarly, for the garlic extract treatment, the size of lesions on twigs decreased from 3.47 before the spray to 3.10, 2.90, 1.80, 1.20, and 0.83 after each successive spray.

For the isolate VK-6B @ 108 cfu/ml and Kokum extract @ 1:5 treatments, the size of lesions on twigs was 4.20 and 3.37 respectively before the initial spray, and it decreased to 4.10, 4.33, 3.80, 4.00, 2.60, 3.90, 2.00, 3.70, and 1.53, 3.53 after the 1st, 2nd, 3rd, 4th, and 5th sprays respectively. However, in the untreated control group, the size of lesions on twigs was 3.23 before the initial spray and increased to 3.43, 4.17, 4.80, 5.00, and 5.33 after each subsequent spray.

Number of lesions per fruit

The highest percentage reduction in the number of lesions per fruit was observed in plants treated with the standard check (Streptocycline @ 500 ppm + COC @ 0.3%), resulting in a significant reduction of 86.12%. This treatment stood out as significantly different from all other treatments. Following this, the garlic extract @ 1:5 treatment demonstrated a reduction of 73.63%. The next most effective treatments were the bio-agent VK-6B isolate @ 108 cfu/ml and kokum extract @ 1:5, which recorded reductions of 61.90% and 51.15% respectively (Table 6 and Fig. 4).

In the standard check treatment, the number of lesions per fruit was 4.00 before the initial spray, and it reduced to 3.67, 3.00, 2.33, 1.67, and 0.55 after the 1st, 2nd, 3rd, 4th, and 5th sprays respectively. Similarly, for the garlic extract treatment, the number of lesions per fruit decreased from 5.89 before the spray to 5.00, 4.67, 3.67, 2.67, and 1.55 after each successive spray.

For the isolate VK-6B @ 108 cfu/ml treatment, the number of lesions per fruit was 3.44 before the initial spray and decreased to 3.33, 3.00, 2.67, 2.33, and 1.22 after the 1st, 2nd, 3rd, 4th, and 5th sprays respectively. Similarly, for plants treated with Kokum extract @ 1:5, the number of lesions per fruit was 4.78 before the initial spray and reduced to 4.33, 4.00, 3.67, 3.00, and 2.33 after each successive spray. However, the untreated control recorded 5.67 lesions per fruit before the initial spray and the number increased to 6.00, 6.33, 6.67, 7.67, and 8.67 after each subsequent spray.

Per cent fruit infection

The highest percentage reduction in fruit infection was observed in the standard check treatment (Streptocycline @ 500 ppm + COC @ 0.3%), resulting in a significant reduction of 81.75%. This treatment outperformed all others, followed by garlic extract @ 1:5 (72.47%). The next most effective treatments were the bio-agent VK-6B isolate @ 108 cfu/ml and kokum extract @ 1:5, which recorded reductions of 57.72% and 48.95% respectively (Table 7 and Fig. 4). In the standard check treatment, the percentage of fruit infection was 45.67% before the initial spray, and it decreased to 41.33%, 38.67%, 29.67%, 18.67%, and 8.33% after the 1st, 2nd, 3rd, 4th, and 5th sprays respectively. Similarly, for the garlic extract treatment, the percentage of fruit infection decreased from 59.33% before the spray to 55.00%, 49.00%, 36.33%, 23.00%, and 16.33% after each successive spray.

For the isolate VK-6B @ 108 cfu/ml treatment and Kokum extract @ 1:5, the initial percentages of fruit infection were 49.67% and 47.67% respectively. These percentages decreased to 47.67%, 44.33%, 43.67%, 40.33%, 38.67%, 35.33%, 33.67%, 30.33%, and 21.00%, 24.33% after the 1st, 2nd, 3rd, 4th, and 5th sprays respectively. However, in the untreated control, the percentage of fruit infection was 41.33% before the initial spray and increased to 43.67%, 47.33%, 49.33%, 51.67%, and 56.67% after each subsequent spray. The yield data were described in Table 7, with the maximum yield observed in the standard check treatment (10.40 t/ha), followed by garlic extract (9.20 t/ha). The isolate VK-6B recorded a yield of 8.00 t/ha, while kokum extract yielded 7.00 t/ha. The least yield (4.20 t/ha) was found in the untreated control. The incremental cost benefit ratio (ICBR) data are also presented in Table 7. All treatments led to an increase in pomegranate yield, with the highest ICBR (1:7.61) observed in the plants sprayed with garlic extract, followed by kokum extract (1:6.13). The standard check treatment recorded the next best incremental cost benefit ratio (ICBR) of 1:5.43.

Discussion

The field trial aimed to effectively manage bacterial blight of pomegranate through the use of bio-agents and botanicals during the period from May to July 2015. The treatments were administered five times at fortnightly intervals and encompassed four distinct bacterial bio-agent isolates (SM-1A, VK-6B, BK-6, and KK-9A) and three botanical extracts (garlic, kokum, and prosopis), alongside a standard check treatment (streptocycline @ 500 ppm + COC @ 0.03%) and an untreated control treated with water spray. The assessment of disease incidence and severity was carried out through various parameters.

The number of lesions exhibited a gradual reduction following each treatment application. Both the standard check and garlic extract displayed notable percentage reductions, with the standard check showing reductions of 6.67%, 26.67%, 46.67%, 73.33%, and 80.00% after the 1st to 5th sprays. Similarly, garlic extract demonstrated reductions of 11.11%, 22.22%, 33.33%, 55.56%, and 77.80% across successive sprays. The isolate VK-6B treatment yielded percentage reductions of 4.67%, 14.29%, 42.86%, 52.38%, and 61.90%, while kokum extract resulted in reductions of 8.33%, 16.67%, 25.00%, 33.33%, and 58.33%.

Regarding leaf infection, the standard check treatment achieved the highest reduction, with reductions of 2.75%, 21.71%, 44.78%, 66.21%, and 83.52% after the 1st to 5th sprays. Following suit, garlic extract showed reductions of 6.15%, 22.91%, 35.48%, 64.81%, and 79.61% in successive sprays. The VK-6B bio-agent isolate exhibited a significant reduction of 74.26%, while kokum extract achieved a reduction of 60.23%.

In terms of leaf area infection, garlic extract emerged as the most effective treatment, showcasing reduction percentages of 10.81%, 22.97%, 41.89%, 62.16%, and 81.08% after each application. The VK-6B bio-agent isolate demonstrated reductions of 5.95%, 11.90%, 35.71%, 54.76%, and 73.81% over successive sprays. Kokum extract yielded promising results as well, with a 70.59% reduction after the 5th spray. This study underscores the efficacy of garlic extract, VK-6B bio-agent isolate, and kokum extract as potent strategies for

effectively managing bacterial blight in pomegranate cultivation.

The most substantial reduction in the number of infected twigs and the size of twig lesions was observed in the standard check treatment, attaining reductions of 76.92% and 78.95%, respectively. However, among the tested botanicals and bio-agent isolates, garlic extract stood out as the most effective. The percentage reduction in the number of infected twigs using garlic extract amounted to 14.29%, 28.57%, 57.14%, and 71.43% following the 1st, 2nd, 3rd, and 4th sprays, respectively. In a similar vein, the reduction in twig lesion size after these spray intervals was 10.58%, 16.35%, 48.08%, and 75.96%. The next promising treatment was the bio-agent VK-6B, yielding reductions of 66.67% in infected twigs and 63.49% in twig lesion size, followed by kokum extract, which achieved reductions of 59.09% in infected twigs and 50.50% in twig lesion size after the 5th spray.

Regarding the number of lesions per fruit and the percentage of fruit infection, the standard check exhibited the highest reduction percentages. After the 5th spray, there was an 86.12% reduction in lesions per fruit and an 81.75% reduction in fruit infection. Garlic extract followed with successive reductions of 15.11%, 20.77%, 37.75%, 54.73%, and 73.63% in the number of lesions per fruit after each spray, while the reduction in fruit infection percentages were 7.30%, 17.42%, 38.76%, 61.24%, and 72.47% after corresponding sprays. Subsequently, the VK-B isolate proved effective with a 61.90% reduction in lesions per fruit and a 57.72% reduction in fruit infection. Kokum extract at a 1:5 dilution achieved a 51.15% reduction in lesions per fruit and a 48.95% reduction in fruit infection after the 5th spray.

In terms of yield, the standard check yielded the highest at 10.40 t/ha, accompanied by an incremental cost-benefit ratio of 1:5.43. Garlic extract followed with a yield of 9.20 t/ha. In contrast, the untreated control only yielded 4.20 t/ha. Rukhsana (2006) [21] demonstrated significant inhibition of bacterial blight in rice using Allium sativum plant extract, achieving a 93.30% disease control rate compared to the control. Similar findings were reported by Akhtar *et al*. (1997) [1], Sinha and Saxena (1999) [22], and Leksomboon *et al*. (2001) [11]. Nine different plant extracts were evaluated at a 10% concentration for managing bacterial blight in pomegranate, with garlic and onion bulb extracts demonstrating the lowest disease incidence and severity after the third treatment (Yenjerappa, 2009) [26].

Basavaraj (2007) [2] showcased the effectiveness of various bio-control agents in managing pomegranate bacterial blight under greenhouse conditions. The alcoholic extract of Garcinia at a 1:5 dilution notably reduced disease severity. Jalaraddi (2006) [8] and Basavaraj (2007) [2] highlighted the efficacy of nine botanical products, including Sufoof-E-Shoneez, in diminishing bacterial blight of pomegranate in field settings. In the current study, garcinia extract emerged as the second-best botanical after garlic extract, showing efficacy against *X. a.* pv. *punicae* both *in vitro* and *in vivo*. The study suggested potential optimization of garcinia extract's acidic nature for further exploration. Field evaluations of different commercial bio-agent formulations against pomegranate bacterial blight demonstrated promising results, particularly with Bacillus subtilis and Pseudomonas fluorescens, achieving a remarkably low incidence of 3.33 PDI (Yenjerappa *et al*., 2013) [25]. Kalita *et al*. (1996) [9] revealed reduced citrus canker disease through the application of phylloplane antagonist species such as *P. fluorescens*, *B. subtilis*, and *B. polymixa*.

Numerous studies have reported the efficiency of bio-agents against *X. oryzae* pv. *oryzae* (Kaur and Thind, 2002; Vasudevan and Gnanamanickam, 2002; Gokil, 2013) [8, 23, 5] under field conditions. The strain KSA1, initially isolated from rhizosphere soil, was tested *in vitro* and in field conditions against *X. citri* subsp. *citri* (Xcc). KSA1 demonstrated efficacy *in vitro* and significantly mitigated symptoms on Mexican lime seedling leaves under field conditions (Mohammed, 2014) [15]. In summary, this study evaluated the field effectiveness of specific bio-agent isolates and botanicals using five fortnightly sprays. The results indicated that garlic extract showed notable efficacy as a botanical treatment, with the bacterial bio-agent isolate VK-6B closely following. Additionally, kokum extract demonstrated potential in reducing both the incidence and severity of bacterial blight in pomegranates.

Table 1: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Number of lesions per leaf after each sprays									
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	5.33	5.33	0.00	5.00	6.25	4.67	12.50	4.33	18.75	4.00	25.00 (29.99)
T_2	VK-6B	10^8 cfu /ml	7.00	6.67	4.76	6.00	14.29	4.00	42.86	3.33	52.38	2.67	61.90 (51.90)
T_3	BK- 6	10^8 cfu /ml	5.00	4.67	6.67	4.33	13.33	4.00	20.00	3.33	33.33	3.00	40.00 (39.24)
T_4	KK- 9A	10^8 cfu /ml	5.00	5.00	0.00	4.67	6.67	4.33	13.33	4.00	20.00	3.67	26.67 (31.09)
T_5	*Garcinia indica*	1:5	4.00	3.67	8.33	3.33	16.67	3.00	25.00	2.67	33.33	1.67	58.33 (50.37)
T_6	*Prosopis juliflora*	1:5	3.33	3.33	0.00	3.00	10.00	3.00	10.00	2.67	20.00	2.33	30.00 (33.21)
T_7	*Allium sativum*	1:5	3.00	2.67	11.11	2.33	22.22	2.00	33.33	1.33	55.56	0.67	77.80 (61.90)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	5.00	4.67	6.67	3.67	26.67	2.67	46.67	1.33	73.33	1.00	80.00 (63.43)
T_9	Untreated control (water spray)		4.67	5.00	-7.14	5.33	-14.29	5.67	-21.43	6.00	-28.57	6.67	-42.86 (40.89)
SEm ±				0.71		0.57		0.66		0.81		0.79	0.56
CD @ 0.05				2.12		1.71		1.97		2.43		2.36	1.68

Note: Figures in parentheses indicate Arc sine transformed values

Table 2: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Per cent leaves infected after each sprays										
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	36.33	35.33 (36.46)	2.75	34.00 (35.67)	6.42	32.67 (34.85)	10.09	30.00 (33.22)	17.43	28.67 (32.36)	21.10 (27.31)
T_2	VK-6B	10^8 cfu /ml	45.33	41.67 (40.20)	8.09	39.33 (38.85)	13.24	26. 00 (30.65)	42.65	19.67 (26.30)	56.62	11.67 (19.97)	74.26 (59.51)
T_3	BK- 6	10^8 cfu /ml	43.67	40.67 (39.62)	6.87	38.67 (38.44)	11.45	37.33 (37.66)	14.50	33.33 (35.27)	23.66	27.00 (31.30)	38.17 (38.15)
T_4	KK- 9A	10^8 cfu /ml	40.00	38.00 (38.06)	5.00	35.33 (36.48)	11.67	33.67 (35.46)	15.83	27.67 (31.74)	30.83	22.67 (28.34)	43.33 (41.16)
T_5	*Garcinia indica*	1:5	29.33	27.67 (31.72)	5.68	25.00 (30.00)	14.77	20.00 (26.56)	31.82	16.00 (23.57)	45.45	11.67 (19.96)	60.23 (50.90)
T_6	*Prosopis juliflora*	1:5	48.00	46.00 (42.71)	4.17	40.33 (39.42)	15.97	34.00 (35.67)	29.17	29.33 (32.79)	38.89	24.33 (29.56)	49.31 (44.61)
T_7	*Allium sativum*	1:5	39.78	37.33 (37.66)	6.15	30.67 (33.63)	22.91	25.67 (30.43)	35.48	14.00 (21.98)	64.81	8.11 (16.43)	79.61 (63.18)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	40.45	39.33 (38.85)	2.75	31.67 (34.24)	21.71	22.33 (28.21)	44.78	13.67 (21.68)	66.21	6.67 (14.95)	83.52 (66.06)
T_9	Untreated control (water spray)		39.11	43.67 (41.35)	-11.65	44.00 (41.54)	-12.50	48.33 (44.04)	-23.58	51.00 (45.58)	-30.40	57.00 (49.03)	-45.74 (42.55)
SEm ±				0.56		0.48		0.34		0.55		0.83	0.73
CD @ 0.05				1.67		1.45		1.04		1.66		2.48	2.20

Note: Figures in parentheses indicate Arc sine transformed values

Table 3: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Per cent leaf area infected after each sprays										
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	32.67	31.67 (34.25)	3.06	29.33 (32.79)	10.20	27.33 31.51)	16.33	25.67 (30.42)	21.43	23.33 (28.87)	28.57 (32.30)
T_2	VK-6B	10^8 cfu /ml	28.00	26.33 (30.88)	5.95	24.67 (29.74)	11.90	18.00 (25.10)	35.71	12.67 (20.80)	54.76	7.33 (15.70)	73.81 (59.22)
T_3	BK- 6	10^8 cfu /ml	28.67	26.00 (30.65)	9.30	25.33 (30.21)	11.63	22.33 (28.17)	22.09	19.00 (25.85)	33.72	14.00 (21.98)	51.16 (45.67)
T_4	KK- 9A	10^8 cfu /ml	36.00	36.00 (37.07)	0.00 p	35.00 (36.27)	2.78	32.67 (34.85)	9.26	29.33 (32.79)	18.52	24.67 (29.78)	31.48 (34.14)
T_5	*Garcinia indica*	1:5	34.00	32.67 (34.82)	3.92	27.33 (31.49)	19.61	20.67 (27.01)	39.22	14.67 (22.51)	56.86	10.00 (18.39)	70.59 (57.17)
T_6	*Prosopis juliflora*	1:5	34.67	30.33 (33.38)	12.50	28.00 (31.93)	19.23	23.33 (28.86)	32.69	20.33 (26.69)	41.35	16.67 (24.08)	51.92 (46.10)
T_7	*Allium sativum*	1:5	24.67	22.00 (27.95)	10.81	19.00 (25.83)	22.97	14.33 (22.22)	41.89	9.33 (17.78)	62.16	4.67 (12.46)	81.08 (64.23)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	29.67	25.00 (29.98)	15.73	20.67 (27.03)	30.34	12.67 (20.78)	57.30	8.00 (16.43)	73.03	3.67 (10.87)	87.64 (69.43)
T_9	Untreated control (water spray)		29.00	31.67 (34.25)	-9.20	36.33 (37.06)	-25.29	38.00 (38.06)	-31.03	41.67 (40.21)	-43.68	48.67 (44.23)	-67.82 (55.44)
SEm ±				0.94		0.85		0.73		0.81		0.81	0.55
CD @ 0.05				2.82		2.53		2.20		2.44		2.43	1.67

Note: Figures in parentheses indicate Arc sine transformed values

Table 4: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Number of twigs infected per plant after each sprays										
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	4.67	4.67	0.00	4.33	7.14	4.33	7.14	4.00	14.29	3.67	21.43 (27.96)
T_2	VK-6B	10^8 cfu /ml	5.00	4.67	6.67	4.00	20.00	3.33	33.33	2.67	46.67	1.67	66.67 (54.73)
T_3	BK- 6	10^8 cfu /ml	6.33	6.00	5.26	5.67	10.53	4.33	31.58	4.33	31.58	3.67	42.11 (40.46)
T_4	KK- 9A	10^8 cfu /ml	6.00	5.67	5.56	5.33	11.11	5.00	16.67	4.67	22.22	4.33	27.78 (31.80)
T_5	*Garcinia indica*	1:5	7.33	7.00	4.55	6.67	9.09	5.00	31.82	4.00	45.45	3.00	59.09 (50.24)
T_6	*Prosopis juliflora*	1:5	7.00	6.67	4.76	6.33	9.52	6.00	14.29	5.00	28.57	4.33	38.10 (38.12)
T_7	*Allium sativum*	1:5	2.33	2.00	14.29	2.00	14.29	1.67	28.57	1.00	57.14	0.67	71.43 (57.71)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	4.33	4.00	7.69	3.33	23.08	2.33	46.15	1.67	61.54	1.00	76.92 (61.33)
T_9	Untreated control (water spray)		5.00	5.33	-6.67	5.67	-13.33	6.33	-26.67	6.67	-33.33	7.33	-46.67 (43.26)
SEm ±				0.47		0.62		0.58		0.43		0.86	0.69
CD @ 0.05				1.41		1.86		1.73		1.28		2.59	2.08

Note: Figures in parentheses indicate Arc sine transformed values

Table 5: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Size of lesions on twigs (cm) after each sprays										
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	2.33	2.33	0.00	2.13	8.57	2.10	10.00	2.07	11.43	2.03	12.86 (21.01)
T_2	VK-6B	10^8 cfu /ml	4.20	4.10	2.38	3.80	9.52	2.60	38.10	2.00	52.38	1.53	63.49 (52.82)
T_3	BK- 6	10^8 cfu /ml	3.60	3.40	5.56	3.20	11.11	2.80	22.22	2.40	33.33	2.13	40.74 (39.67)
T_4	KK- 9A	10^8 cfu /ml	4.40	4.33	1.52	4.00	9.09	3.90	11.36	3.70	15.91	3.53	19.70 (26.33)
T_5	*Garcinia indica*	1:5	3.37	3.20	4.95	3.00	10.89	3.20	4.95	2.30	31.68	1.67	50.50 (45.29)
T_6	*Prosopis juliflora*	1:5	4.67	4.24	9.14	3.96	15.14	3.52	24.57	3.21	31.21	3.00	35.71 (36.69)
T_7	*Allium sativum*	1:5	3.47	3.10	10.58	2.90	16.35	1.80	48.08	1.20	65.38	0.83	75.96 (60.65)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	3.17	3.00	5.26	2.50	21.05	1.50	52.63	1.00	68.42	0.67	78.95 (62.70)
T_9	Untreated control (water spray)		3.23	3.43	-6.19	4.17	-28.87	4.80	-48.45	5.00	-54.64	5.33	-64.95 (53.70)
SEm ±				0.18		0.42		0.31		0.54		0.51	0.24
CD @ 0.05				0.53		1.27		0.94		1.62		1.55	0.72

Note: Figures in parentheses indicate Arc sine transformed values

Table 6: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Number of lesions per fruit after each sprays										
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction
T_1	SM- 1 A	10^8 cfu /ml	7.11	7.00	1.59	6.67	6.28	6.33	10.97	5.67	20.34	5.00	29.71 (33.02)
T_2	VK-6B	10^8 cfu /ml	3.44	3.33	3.19	3.00	12.88	2.67	22.56	2.33	32.24	1.22	64.47 (53.42)
T_3	BK- 6	10^8 cfu /ml	6.00	5.67	5.56	5.33	11.11	4.67	22.22	4.00	33.33	3.44	42.61 (40.74)
T_4	KK- 9A	10^8 cfu /ml	7.11	7.00	1.59	6.67	6.28	5.67	20.34	5.33	25.02	4.89	31.26 (33.98)
T_5	*Garcinia indica*	1:5	4.78	4.33	9.28	4.00	16.26	3.67	23.24	3.00	37.19	2.33	51.15 (45.65)
T_6	*Prosopis juliflora*	1:5	5.00	4.67	6.73	4.33	13.39	4.00	20.05	3.67	26.72	3.00	39.97 (39.21)
T_7	*Allium sativum*	1:5	5.89	5.00	15.11	4.67	20.77	3.67	37.75	2.67	54.73	1.55	73.63 (59.11)
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	4.00	3.67	8.33	3.00	25.00	2.33	41.67	1.67	58.33	0.55	86.17 (68.18)
T_9	Untreated control (water spray)		5.67	6.00	-5.88	6.33	-11.76	6.67	-17.65	7.67	-35.29	8.67	-52.94 (46.69)
SEm ±				0.49		0.49		0.56		0.37		0.99	0.56
CD @ 0.05				1.45		1.48		1.67		1.10		2.97	1.68

Note: Figures in parentheses indicate Arc sine transformed values

Table 7: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate

Treatments	Treatment details	Concentration	Per cent fruit infection after each sprays											Yield		ICBR
			Before spray	1st	% reduction	2nd	% reduction	3rd	% reduction	4th	% reduction	5th	% reduction	Fruits/ plant	t/ha	
T_1	SM- 1 A	10^8 cfu /ml	33.67	32.33 (34.63)	3.96	31.33 (34.02)	6.93	30.33 (33.36)	9.90	29.00 (32.59)	13.86	27.00 (31.29)	19.80 (26.42)	28	5.60	1:2.45
T_2	VK-6B	10^8 cfu /ml	49.67	47.67 (43.66)	4.03	43.67 (41.35)	12.08	38. 67 (38.44)	22.15	33.67 (35.47)	32.21	21.00 (27.28)	57.72 (49.44)	40	8.00	1:3.12
T_3	BK- 6	10^8 cfu /ml	47.33	45.33 (42.32)	4.23	41.00 (39.81)	13.38	39.67 (39.03)	16.20	36.00 (36.87)	23.94	31.67 (34.23)	33.10 (35.11)	30	6.00	1:1.48
T_4	KK- 9A	10^8 cfu /ml	41.67	40.67 (39.62)	2.40	39.33 (38.83)	5.60	37.00 (37.47)	11.20	34.67 (36.07)	16.80	32.33 (34.66)	22.40 (28.24)	29	5.80	1:1.31
T_5	*Garcinia indica*	1:5	47.67	44.33 (41.74)	6.99	40.33 (39.42)	15.38	35.33 (36.48)	25.87	30.33 (33.41)	36.36	24.33 (29.56)	48.95 (44.39)	35	7.00	1:6.13
T_6	*Prosopis juliflora*	1:5	35.00	33.67 (35.46)	3.81	29.67 (33.00)	15.24	27.67 (31.74)	20.95	26.00 (30.65)	25.71	24.00 (29.34)	31.43 (34.09)	31	6.20	1:2.33
T_7	*Allium sativum*	1:5	59.33	55.00 (47.87)	7.30	49.00 (44.42)	17.42	36. 33 (37.06)	38.76	23.00 (28.65)	61.24	16.33 (23.83)	72.47 (58.35)	46	9.20	1:7.61
T_8	Standard check (Streptocycline + COC)	500 ppm +0.3%	45.67	41.33 (40.01)	9.49	38.67 (38.44)	15.33	29. 67 (33.01)	35.04	18.67 (25.55)	59.12	8.33 (16.60)	81.75 (64.72)	52	10.40	1:5.43
T_9	Untreated control (water spray)		41.33	43.67 (41.37)	-5.65	47.33 (43.47)	-14.52	49.33 (44.61)	-19.35	51.67 (45.95)	-25.00	56.67 (48.84)	-37.10 (37.52)	21	4.20	
SEm ±				0.74		0.56		0.72		0.50		0.70	0.46			
CD @ 0.05				2.21		1.69		2.16		1.51		2.11	1.38			

*ICBR-Incremental Cost Benefit Ratio

Note: Figures in parentheses indicate Arc sine transformed values

Fig. 1a

Fig. 1b

Fig 1c

Fig. 1d

Fig. 1e

Fig 1: *In vivo* evaluation of bio-agents and botanicals against bacterial blight of pomegranate. (1a) Field view of trial conducted to test the efficacy of bio-agents and botanicals on bacterial blight of pomegranate, Sokanadagi, Bagalkot-2015; (1b) Garlic extract @ 1:5; (1c):VK-6B @ 108 cfu/ml; (1d) Streptocycline + COC (Standard check); (1e) Untreated control

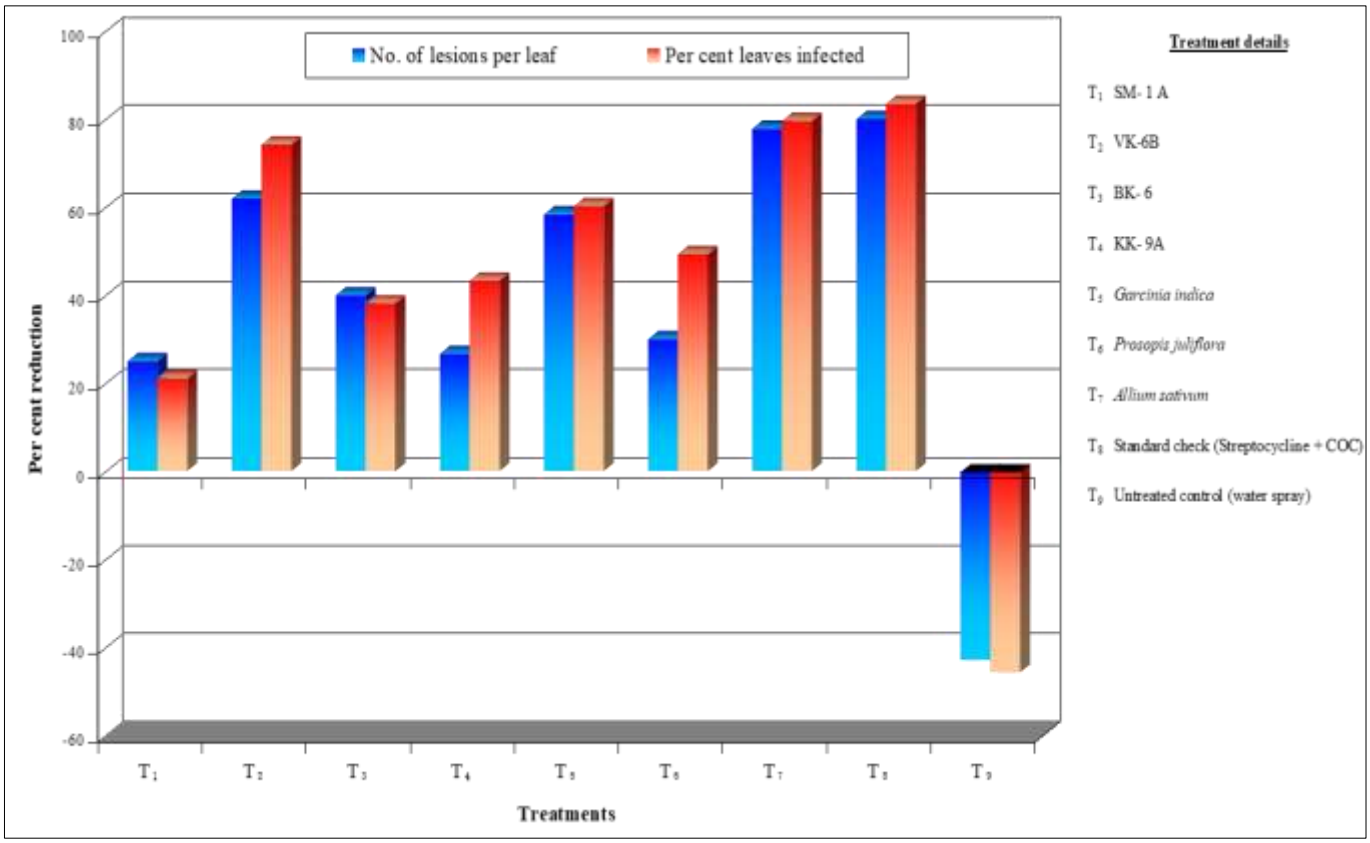

Fig 2: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate on leaf

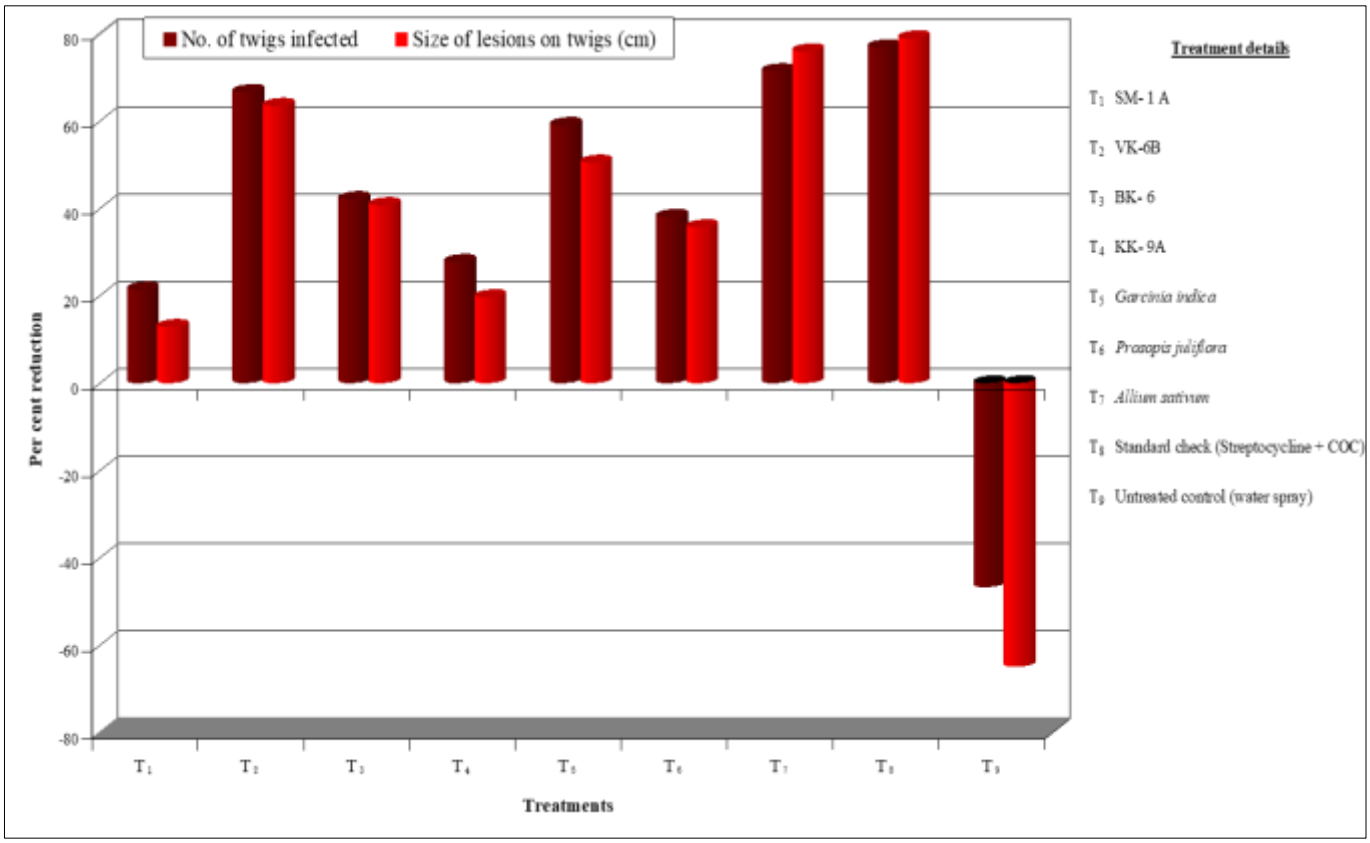

Fig 3: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate on twig

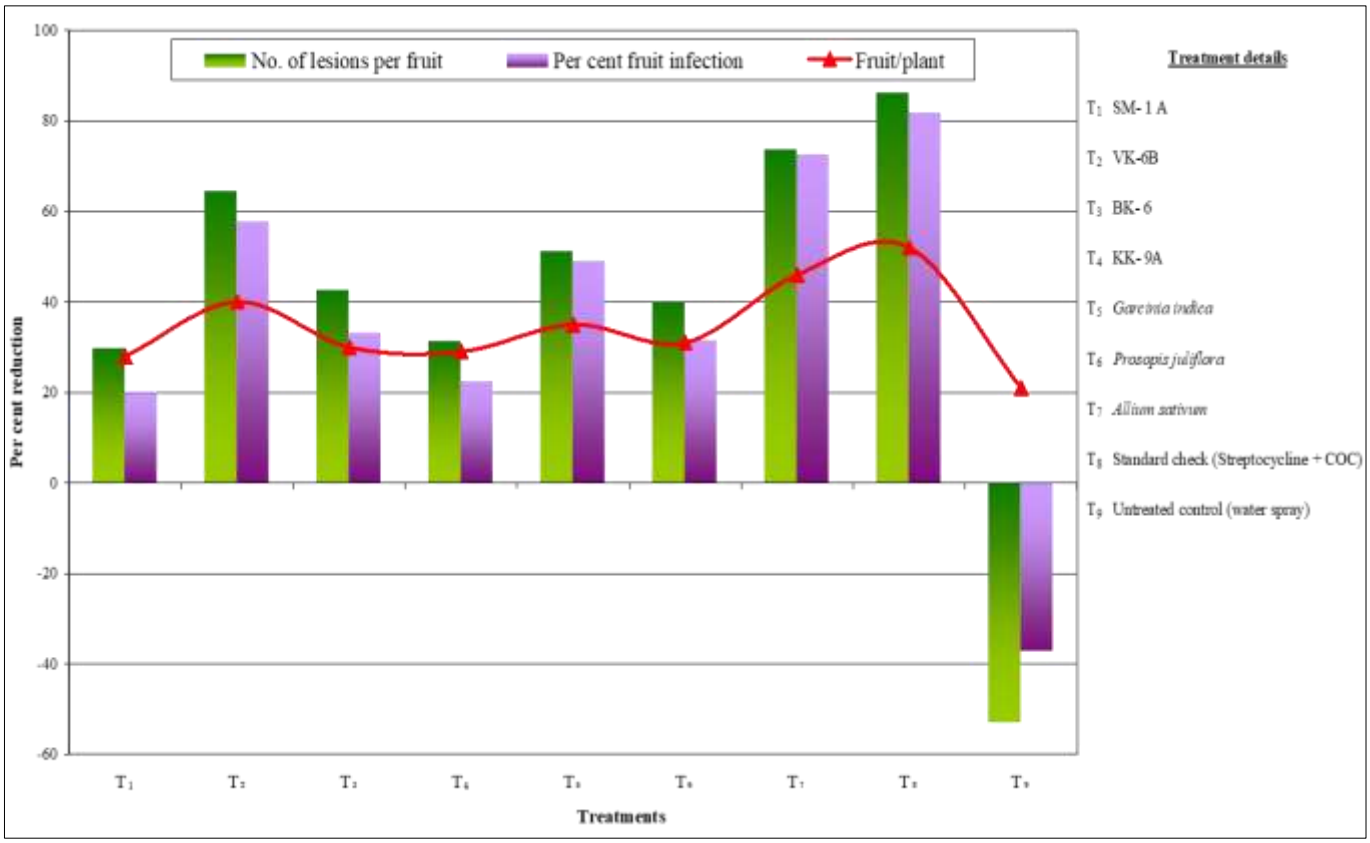

Fig 4: Effect of plant extracts and bio-agents on development of bacterial blight of pomegranate on fruits

Conclusion

This study conducted to assess the efficacy of diverse botanical extracts and bacterial antagonists against pomegranate bacterial blight in Bagalkot, Karnataka, India. Notably, applying garlic (*Allium sativum*) extract at a 1:5 dilution, administered five times fortnightly, displayed exceptional disease control. Significant reductions were observed, with 77.80% inhibition for lesions per leaf, 79.61% for leaf infection, 81.08% for leaf area affected, 71.43% for twig infection, 75.96% for twig lesion size, 73.63% for lesions per fruit, and 72.47% for fruit infection. While garlic proved effective, the standard Streptocycline and COC treatment exhibited the highest potency, achieving substantial reductions: 80.00% for lesions per leaf, 83.52% for leaf infection, 87.64% for leaf area affected, 76.92% for twig infection, 78.95% for twig lesion size, 86.17% for lesions per fruit, and 81.75% for fruit infection. Additionally, kokum (Garcinia indica) fruit extract at a 1:5 dilution emerged as a noteworthy botanical treatment, delivering significant percentage reductions across key disease parameters. Furthermore, the native VK-6B strain of *Pseudomonas fluorescens* exhibited remarkable disease suppression, with notable percentage reductions in various disease aspects. The antibacterial mechanisms were attributed to specific bioactive compounds, such as garlic extract's 8-amino (8-butoxyoct) -2-(enyldisulfanyl) -3- (2-hydroxy-4-methoxy phenyl) prop-1-enyl benzene-1,3-diol, and kokum (*G. indica*) fruit extract's Garcinol. These findings highlight the potential of VK-6B, garlic, and kokum extracts as eco-friendly solutions against pomegranate bacterial blight, calling for further research to uncover active constituents for refined disease management strategies.

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