

MANAGEMENT OF APRICOT SHOT HOLE DISEASE (*Wilsonomyces carpophilus*) USING TRUNK INJECTION DELIVERY SYSTEM

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ABSTRACT

Shot-hole disease (SHD) is a widely spread and devastating disease of apricots that leads to severe yield loss and poor quality of fruit. However, limited research has been conducted on SHD management using trunk injection delivery systems in Gilgit-Baltistan, Pakistan. In this study, the efficacy of individual (Bordeaux mixture (BM), *Capparis spinosa* (CP), *Mentha piperita* (MP), *Artemisia vulgaris*; (AV) and in combination (BM + MP, BM + CP, BM + AV) of BM and plant extracts treatments were evaluated against SHD. The experiment was conducted in the apricot orchards during 2022-23 as a complete randomized block design under factorial arrangement. The analysis of variance showed significant ($P \leq 0.5$) variation in SHD incidence among the treatments. Among individual treatments, BM was the most effective against SHD with 27.4% incidence, followed by AV (39.8%), whereas CP and MP were moderately efficacious (means: 43.03% and 43.14%, respectively). The control treatment showed extremely high disease occurrence (73.77%). Moreover, the highest effectiveness was found in the combination treatments. BM + MP reduced the disease incidence rate to 29.67%, followed by BM + CP (47.32%), which provided a more stable form of disease suppression. Hence, BM alone and in combination with plant extracts effectively reduce SHD. These results highlight the potential of plant-based trunk injection treatments for SHD management, with BM + MP offering the most effective control.

Keywords: Apricot; Bordeaux mixture; cultivars; plant protective material; shot hole disease; trunk injection

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INTRODUCTION

Apricot (*Prunus armeniaca* L.) is a widely cultivated and significant fruit crop worldwide, serving as a key agricultural product of local economies, with numerous health benefits. The production of apricots is concentrated in several key countries worldwide, with Turkey, Iran, Uzbekistan, Italy, and the United States leading the way in terms of production volume. According to the Food and Agriculture Organization (FAO, 2020), apricots contribute to the livelihoods of millions of farmers worldwide. They offer nutritional benefits, such as high levels of vitamins A and C serve as an essential ingredient in many food products (USDA, 2020; Chen *et al.*, 2020). Apricots are one of the major fruits in Pakistan, with around 300,000 tons produced annually, primarily in Gilgit-Baltistan (GB) and Balochistan. About 96% of the income from apricot sales goes to small farmers, supporting sustainable livelihoods and poverty alleviation in these regions (PBS, 2023). However, apricot is severely affected by various diseases, including crown gall, gummosis, *Phytophthora* root and

crown rot, collar rot, brown rot, and shot hole disease, which significantly reduces yields and causes substantial economic losses (Abbas *et al.*, 2023). It is caused by a fungus known as *Wilsonomyces carpophilus*, which is most destructive worldwide, including GB. Several studies have reported high prevalence (>70%), incidence (>40%), and severity (>25%) of the disease in dominant apricot-producing valleys of GB (Azhar *et al.*, 2023; Muhammad *et al.*, 2022). SHD manifests as spots on apricot fruit and leaves in spring (April to June), with severe cases leading to leaf drop. Fruit symptoms include light brown lesions with dark purple margins, often clustered on the upper side of the fruit (Saleem *et al.*, 2020). Managing apricot shot hole disease has been challenging due to varying canopy sizes, which reduce the effectiveness of disease control methods. In GB, apricot growers rely on chemical spray using handheld sprayers, but this method is often ineffective, as studies show that up to 70% of the protective material drifts into the environment. At the same time, negative impacts are borne on human health, the environment, and the economy by the marginalized farming community. To

address this issue, the trunk injection system offers a more targeted, cost-effective, and environmentally friendly solution. This method directly delivers treatment into the tree's vascular system, reducing pesticide drift and ensuring active ingredients reach the infection site (Hu *et al.*, 2018; Archer *et al.*, 2022). Hence, the objective of this study is to manage SHD in apricots using a trunk injection delivery system. Trunk injection minimizes pesticide spray, thereby reducing pesticide waste and risks to non-target organisms, such as beneficial insects (Werrie *et al.*, 2021). The current state of knowledge regarding the control of apricot shot hole disease (*Wilsonomyces carpophilus*) in the region, particularly within agricultural research and extension departments, is minimal. Traditional control methods, such as foliar fungicide sprays and cultural practices, are commonly used but often fail to provide effective and long-lasting management. There is a significant knowledge gap in exploring innovative, precise, and environmentally friendly disease control strategies. This study introduces trunk injection technology as a method to control shot hole disease in apricots. The novel aspect of this research lies in its targeted application, which minimizes environmental contamination and ensures efficient uptake of fungicides directly into the tree's vascular system. If successful, this technology could be extended to other fruit crops, such as apples and grapes, as well as for the control of various pests, offering a new and sustainable approach to tree health management.

MATERIALS AND METHODS

Study area: The experiment was conducted in Gilgit District, GB, from 2022 to 2023 to manage SHD in apricot trees. A high disease incidence was observed during the 2020-21 field survey (Azhar *et al.*, 2023). Prior to applying treatments via trunk injection, consent was obtained from the orchard owners.

Experimental design: In 1st experiment, water extracts of individual treatments (B1) such as *Capparis spinosa* (SP), *Mentha piperita* (MP), *Artemisia vulgaris* (AV), and Bordeaux Mixture (BM) were injected into the trunk to manage SHD on three apricot cultivars viz. Habi, Shakanda and Halman. However, water was injected as a control. This experiment was laid out following a randomized complete block design (RCBD) under factorial arrangement and replicated three times. In 2nd experiment, BM was combined with every plant extract at a 50:50 ratio (BM + SP, BM + MP, BM + AV) along with water (as control) as combination treatments (B2) injected in the above-mentioned apricot cultivars to manage SHD. The 2nd experiment was also RCBD with factorial arrangement and replicated three times. About 10 ml of each protective agent (BM, SP, MP, AV) was

applied to three trunk ports in each apricot tree. Each treatment was applied to three trees per replication.

Preparation of plant extracts: Fresh leaf samples from three medicinal plants were collected in the vicinity of District Gilgit and brought to the Laboratory of the Department of Agriculture and Food Technology, Karakoram International University Gilgit, Gilgit-Baltistan, Pakistan. Samples were thoroughly washed under running water, submerged for 20 minutes in a 2% sodium hypochlorite solution, and then rinsed thoroughly with distilled water prior to air drying at room temperature. After drying, the leaves were milled into a fine powder. A total of 100 g of each finely ground dry plant material was extracted in 100 ml of ethanol following the method described by Neela *et al.* (2014). The solution was allowed to stand at room temperature for the entire night prior to filtering using Whatman filter paper No. 1.

Preparation of Bordeaux mixture: BM was prepared by combining copper sulfate, lime, and distilled water at a ratio of 1:1:100 (1 kg CuSO₄, 1 kg Ca (OH)₂, and 100 liters of H₂O).

Development of trunk injection port: The apricot trunk injection port was developed by following the procedures reported by Acimovic *et al.* (2015). Firstly, the circumference of the tree trunk was measured in inches to determine diameter at breast height (DBH). Then, the obtained value was divided by 3.14 to obtain DBH in inches. Now, the number of injection sites was determined by dividing DBH by 2. For individual treatment, 10 mL of the treatment was injected into each port. The number of ports on each apricot tree was calculated as described by Acimovic *et al.* (2015). For the combined treatment, the amount of protective material injected was determined based on the number of ports. Of the total, 50% was made up of selected plant-based biopesticides, and the remaining 50% was the BM.

The efficacy of the plant protective material against SHD was evaluated by determining the incidence in each apricot fruit. Treatments were applied to each tree based on the disease incidence observed in marked trees during the field survey. The percentage of disease incidence reduction after treatments was calculated using the formula given below (Arafat *et al.*, 2012).

$$\text{DIR (\%)} = \frac{\text{DIMIT} - \text{DITT}}{\text{DIMIT}} \times 100$$

DIR (%): Disease incidence reduction percentage; DIMIT: Disease incidence marked trees; DITT: Disease incidence treated trees.

Data analysis: A two-way ANOVA was performed to evaluate the significance of individual and interactive effects in both experiments using Statistix 8.1. In case of significant effect, the means were compared using the Least Significant Difference (LSD) at $p \leq 0.05$.



Figure 1. Field activities to apply trunk injection technology in apricot orchards

RESULTS

Experiment 1: Effect of individual plant water extracts and Bordeaux mixture on SHD disease incidence in different apricot cultivars: The analyzed data indicated that trunk injected individual plant water extracts and Bordeaux mixture (T), apricot cultivars (C) and interaction among them (T×C) had a significant effect on SHD incidence in apricot (Table 1). Trunk injected plant water extracts and Bordeaux mixture (BM) had significantly lowered the SHD incidence in all tested apricot cultivars compared with control. The control

treatment recorded the highest mean values of SHD incidence for all three varieties, i.e., Habi (78.22), Shakanda (70.88), and Halman (72.22), compared with plant water extracts and BM. Nonetheless, trunk-injected BM recorded the lowest SHD incidence in all three cultivars (Habi: 25.77, Shakanda: 24.88, Halman: 31.54). Moreover, the trunk-injected plant water extracts lowered the SHD incidence compared with only control in all tested cultivars; however, they were not compared with BM application. In general, the results indicate that BM, compared to plant extracts, significantly reduced more SHD incidence (Table 2).

Table 1. ANOVA table for the individual plant protection treatments and cultivars effect on SHD incidence in apricot

Sources of variations	Sum of Squares	df	Mean Square	F-value	P-value
Treatments (T)	13097.69	4	3274.42	185.42	0.000**
Cultivars (C)	117.62	2	58.81	3.33	0.050*
Interaction (T × C)	465.01	8	58.13	3.29	0.009**
Error	494.47	28	17.66		

df: Degree of freedom; **: Significant at p≤0.01; *: Significant at p≤0.05

Table 2. Effect of individual plant protection treatments on shot hole disease incidence of different apricot cultivars.

Treatments	Habi	Shakanda	Halman
Control	78.22 a	70.88 a	72.22 a
BM	25.77 ef	24.88 f	31.54 d-f
<i>C. spinosa</i> (CP)	39.10 b-d	40.65 a-d	49.33 a
<i>M. piperita</i> (MP)	44.22 a-c	43.88 a-c	41.33 a-d
<i>A. vulgaris</i> (AV)	35.77 c-e	37.54 b-d	46.22 ab

Means, within the same column or row, followed by the same letter (s) are not significantly different at p≤ 0.05

Experiment 2: Effect of combination application of plant water extracts and Bordeaux mixture on SHD disease incidence in different apricot cultivars:

The analyzed data indicated that the occurrence of SHD in apricots was significantly influenced by combination treatments (CT), apricot cultivars (C), and their interaction (CT × C) (Table 3). The CTs significantly reduced SHD incidence across the three cultivars compared to the control treatment. The control treatment had the highest SHD incidence as compared to combination treatments (Table 4). Among the combination treatments, the BM and MP (Bordeaux mixture + *M. piperita*) combination significantly lowered the SHD incidence in all tested apricot cultivars, with

mean values of 25.32, 28.22, and 35.48, respectively. The second combined treatment that had significantly reduced SHD incidence compared to the control was BM + AV (Bordeaux mixture + *Aloe vera* extract), with average values of 39.32, 40.00, and 48.22 for the cultivars Habi, Shakanda, and Halman, respectively. Also, BM + CP (Bordeaux mixture + *Calotropis spinosa*) reduced SHD incidence compared to control and was lower to an extent between BM + MP and BM + AV, with recorded mean values of 46.66, 45.10, and 50.22 in the cultivars of Habi, Shakanda, and Halman respectively. The results indicate that overall, BM + MP was the best combination treatment in order to reduce SHD incidence in all apricot cultivars tested (Table 4).

Table 3. ANOVA table to check the significance of cobined application of plant water extracts with BM and cultivars effect on SHD incidence in apricot

Source of variations	Sum of Squares (SS)	df	Mean Square (MS)	F-value	P-value
Combination Treatment (CT)	9868.46	3	3289.49	169.17	0.000**
Cultivar (C)	364.68	2	182.34	9.38	0.001**
Treatment × Cultivar (CT × C)	504.23	6	84.04	4.32	0.005*
Error	427.79	22	19.44		

df: Degree of freedom; **: Significant at $p \leq 0.01$

Table 4. Effect of combined application of plant water extracts and BM on SHD incidence in apricot cultivars.

Treatments	Habi	Shakanda	Halman
Control	78.22 a	70.88 a	72.22 a
BM + MP	25.32 d	28.22 d	35.48 c
BM + CP	46.66 ab	45.10 b	50.22 a
BM + AV	39.32 c	40.00 c	48.22 ab

Means, within the same column or row, followed by the same letter (s) are not significantly different at $p \leq 0.05$

Here BM: Bordeaux mixture; MP: *M. piperita*; CP: *C. spinosa*; AV: *A. vulgaris*

DISCUSSION

In this study, trunk injection of plant-based treatments was very effective against the SHD incidence, especially among apricot cultivars. Among all the individual treatments, BM appeared to be the most potent in suppressing the disease, indicating its great scope for use as a possible biocontrol agent. These findings corroborate several studies that reveal the utility of BM in plant disease management through their antimicrobial properties and improving the host resistance (; Hu *et al.*, 2018; Archer *et al.*, 2022; Benigno *et al.*, 2024). In contrast, moderate effects were exhibited by *C. spinosa* and *M. piperita*. Similar performance of these two treatments suggests the interplay of a common mechanism, either through antifungal activity or induced resistance in the host plant. The use of combination treatments in experiments was revealed to play a role above and beyond either alone, with BM + MP being rated the best treatment. This implies that the mixture of biomaterial with plant extracts induces a combined effect

that boosts antimicrobial activities along with more substantial host resistance (Acimovic, 2015). BM + CP also showed promising protection outcomes, which translated to consistent performance over various varieties. However, BM + AV offered only moderate efficacy but was mostly variable tending, suggesting that while it does cause suppression of the disease, its performance is not stable across apricot cultivars. The estimations present significantly different results among treatments, with individual and combination treatments working effectively compared to the control. Here, the effect of replication was not statistically significant ($P = 0.260$), thereby elucidating the reproducibility of the entire work since the differences that took place were due to the treatments only (Archer *et al.*, 2022; Hue *et al.*, 2018). This study underlines a number of major advantages of trunk injection over traditional foliar spraying techniques for the delivery of protective substances. According to Acimovic (2015), conventional ground sprayers are not very effective in pesticide delivery, and as little as 0.4% reaches the targeted pest.

Air blast sprayers are also inefficient at getting materials to their target (Zhu *et al.*, 2006). Only 29 to 56% of the applied spray solution settles on the tree canopy, with the remainder drifting to the ground or non-target areas. Pesticide drift poses significant threats to ecosystems, contaminating air, water, and soil and harming non-target organisms like pollinators and aquatic life. Such drift can disrupt biodiversity and ecosystem balance, leading to long-term ecological damage (Mineau and Whiteside 2013). Effective mitigation strategies are imperative to safeguard the environmental health and economic use of the pesticide. In the current study, a BM and plant-based biopesticide were used to manage SHD in field conditions. The efficacy of BM against fruit diseases has been well-documented. Previous studies (Dang *et al.*, 2017; Patil *et al.*, 2023; Mudassir *et al.*, 2019) have demonstrated their effectiveness in managing various pathogens, reducing disease incidence, and improving fruit quality. Bordeaux offers a promising solution for disease management in fruit orchards, contributing to higher yields and healthier crops. In the present study, plant-based bio-pesticides (*Capparis spinosa*, *Mentha piperita* and *Artemisia vulgaris*) were used to fight SHD. Results revealed that these extracts showed high efficacy in managing the disease incidence in the field. The study suggests that these extracts can be utilized effectively to reduce disease incidence and insect infestation as alternatives to chemical pesticides. These findings also align with previous studies on the genus *Mentha*, which exhibits high biopesticide activity due to its fungicidal and insecticidal potential (Singh and Pandey, 2018; De Clerck *et al.*, 2020). *C. spinosa* has been tested previously against fungal growth (Rajhi *et al.*, 2021; Benzidane *et al.*, 2020; Al-Askar, 2012) which exhibited its effectiveness in inhibiting disease development. Compounds (phenols, tannins, steroids, flavonoids, fatty acids, saponin, terpenoids, alkaloids, and volatile oils) present in *C. spinosa* offer potential as natural alternatives for fungal diseases. Plant extracts of *A. vulgaris* have also demonstrated efficacy against shot hole fungus in all observed apricot cultivars during the study. *A. vulgaris* has shown notable efficacy against plant diseases. The fungal efficacy of the selected plant (Singh *et al.*, 2023; Obistoiu *et al.*, 2014). Several researchers have identified compounds such as sesquiterpene lactones, flavonoids, and essential oils that possess strong antifungal activities (Andreu *et al.*, 2018; Sepahvand *et al.*, 2017).

Conclusion: In conclusion, SHD was managed using a trunk injection delivery system. Bordeaux mixture alone and combined with plant extracts were effective in managing SHD compared to other treatments. Overall, BM + MP shows the highest efficacy among the treatments tested and must, therefore, be considered a promising alternative to traditional control approaches.

Future studies should optimize trunk injection work, study the long-term influences of these treatments on tree health and yield, and determine their sustainability for the environment. Carrying out additional field trials in various climates and incorporating other bioactive plant compounds might certainly improve management strategies for SHD in apricot orchards.

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