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Effectiveness of GRAS substances for managing leaf diseases in brinjal (*Solanum melongena* L)

R. **Thibisha* and K. Prasannath**

Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka

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Abstract

Leaf diseases in brinjal (*Solanum melongena* L.) are common and drastically lower its yield. As synthetic fungicides are hazardous to the environment and human health, non-fungicidal approaches to foliar disease control are being adopted progressively. The current investigation assessed the effectiveness of Generally Recognized as Safe (GRAS) substances in the management of leaf diseases in brinjal. The efficacy of the selected GRAS substances, including 400 µM of salicylic acid, 0.1% of sodium bicarbonate, and botanical extracts such as 200 ppm of garlic clove extract, 200 ppm of neem extract and 200 ppm of eucalyptus extract, were assessed. Six treatments and four replicates were employed in a completely randomized design for this present study. The natural occurrence of leaf diseases was taken into account in the experiment. Disease incidence (%), disease severity (%), number of leaves per plant, plant height (cm) and number of branches per plant were recorded seven weeks after

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*Corresponding author
Postal address: Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka, Palachcholai, Kaluwankerny-30372
Email: thibithibi26@gmail.com

transplanting. Analysis of variance was used to assess the significance of treatment effects. The findings confirmed that the exogenous spraying of salicylic acid and garlic clove extract significantly ($P<0.05$) decreased the disease severity and incidence, with statistically equivalent efficiency for both substances. Moreover, number of branches per brinjal plant (3.3), plant height (51.4 cm) and number of leaves per brinjal plant (31.5) were significantly ($P<0.05$) enhanced by salicylic acid compared to control. Hence, spraying garlic clove extract and salicylic acid may therefore be an environmentally potential means of managing brinjal leaf diseases during the vegetative growth stage.

Keywords: botanicals, eco-friendly disease management, garlic clove extract, leaf diseases, salicylic acid, systemic acquired resistance.

Introduction

The brinjal (*Solanum melongena* L.) is a widely consumed vegetable all over the world (Gürbüz *et al*., 2018). Brinjal seriously suffers from various diseases, among those fungal infections that cause more than 50% of the losses in brinjal production as fungal diseases are one of the most dangerous biological stresses that seriously harm agricultural crops (Tsitsigiannis *et al*., 2008). Especially, fungal leaf diseases are more prevalent in warm climatic regions like Sri Lanka, where stable warmth and comfortable humidity are typical (DOA, 2022). Loss of crops and lower yields are mainly caused by systemic leaf diseases like leaf spots and leaf blight (Iqbal *et al*., 2018). They indirectly impact pod quality formation because they alter crop transpiration and photosynthetic rates (Ponmurugan *et al*., 2007). To tackle certain foliar diseases, agrochemical products are typically employed. The application of synthetic fungicides to plant materials, particularly food crops, has drawn criticism, though, as these compounds have a lengthy half-life, and can leave residual toxicity that could be hazardous to human health and can contaminate the environment (Kalupahana *et al*., 2020). Consequently, the development of chemical-free alternatives to synthetic fungicides is essential. The use of Generally Recognized as Safe (GRAS) substances in place of synthetic fungicides has garnered more attention in recent years. These natural antimicrobial agents are non-permanent, non-toxic, and renewable. With no threat to the ecosystem or human health, GRAS's

effective antimicrobial properties stimulate plant defenses against many kinds of plant diseases (Elsalam *et al.,* 2017). Thus, the aim of this experiment was to examine the effectiveness of various GRAS substances in managing leaf diseases in brinjal plant under field conditions.

Materials and Methods

Experimental site

The assessment was undertaken at the crop farm, Faculty of Agriculture, Palachcholai, Eastern University, Sri Lanka is situated at an elevation of 7 m above mean sea level in the latitude 7° 48' 30" N and the longitude 81° 35' 6" E, with the temperature ranges from 30 °C to 32 °C and mean annual rainfall from 1400 mm to 1680 mm. It is affiliated to the agro-ecological zones of the low country dry zone in Sri Lanka, with sandy regosol soil. The experiment was carried out during the time frame of August to November, 2022.

Collection of brinjal seedlings

Healthy, vigorous two-week-old seedlings of the brinjal variety "Thinnaveli purple" were collected from the nursery unit of the Crop Farm of the Faculty of Agriculture, Eastern University, Sri Lanka, for the research.

Preparation of poly-bags with media and planting

This experiment was conducted using polybags of size 40 cm × 20 cm under *in vivo* conditions. The seedlings were grown in polybags at a ratio of 1:1 using a mixture of topsoil and cow dung (Department of Agriculture, Sri Lanka, 2022). Two-week-old, uniform, healthy brinjal seedlings were selected for transplanting, and each seedling was planted in each polybag to achieve one plant per polybag.

Experimental design

The investigation was done in a completely randomized design (CRD) with six treatments and four replicates. The young sprouted plants were exposed to natural infestation from foliar diseases.

Treatment structure

T_1 – 0.1% of sodium bicarbonate, T_2 – 400 µM of salicylic acid, T_3 – 200 ppm of neem leaf extract (*Azadirachta indica)*, T_4 – 200 ppm of eucalyptus leaf extract (*Eucalyptus globulus)*, T_5 – 200 ppm of garlic clove extract (*Allium sativum)* and T_6 – Control (No application).

Preparation of GRAS compounds

For the botanical extract preparation, a sufficient quantity of each plant's corresponding healthy portions were obtained to prepare the botanical extracts. The plant portions were chopped, dried in the sun, and pulverized to make dehydrated powders. Around 20 g of dry powder from each plant portion was well extracted over a period of six hours using 300 mL of 99.8% methanol in a Soxhlet apparatus. The methanol containing botanical extracts were routed to rotary evaporation and eventually condensed in a vacuum at a temperature of 40 °C under reduced pressure. The extracted liquids were kept in sealed bottles at room temperature (30±2 °C) after being further dehydrated to dryness by shade drying (Gopalasatheeskumar, 2018). In accordance with their weights, dehydrated solid particles of botanical extracts were diluted to the proper proportions by incorporating distilled water. A concentration of 200 ppm of each botanical extract was adopted for spraying, and they were prepared by combining the proper quantity of stock solution of above-mentioned botanical extracts with distilled water. For the sodium bicarbonate and salicylic acid solution preparation, required amount of powdered sodium carbonate and salicylic acid were measured and brought into the volume of 240 mL with distilled water to attain the concentration of 0.1% and 400 µM, respectively. Prior to the addition of distilled water, salicylic acid powder was dissolved in 1 mL of absolute ethanol for better dissolving.

Spraying of GRAS compounds

The foliar spraying was started two weeks after transplanting during their vegetative growth and it was done three times at fortnight intervals. Plant extracts, sodium bicarbonate solution and salicylic acid solutions were applied as foliar sprays in the quantity of 240 mL to each of the treatments. The spraying was done by a hand sprayer to cover the entire surface of plant leaves. An amount of 60 mL solution was sprayed on one plant. Foliar spraying was carried out during the late evening and precautions were taken to prevent the drifting of spray components to neighboring plants.

Identification of leaf diseases in brinjal plant

According to disease diagnostic guidelines and information from past studies (Salih and Abdulridha, 2019), types of leaf diseases were visually recognized based on the characteristic symptoms displayed by the corresponding diseases.

Data collection

Disease parameters

Data on the severity and incidence of the disease were measured from the brinjal foliage exhibiting symptoms of leaf disease for each treatment. The fungal diseases that caused the spots and blights were verified based on their distinctive symptoms. Data recordings were done seven weeks following the transplanting. The number of foliages of each plant exhibiting characteristic symptoms of leaf diseases was noted, and the following formula was used to determine the incidence of leaf diseases (Marolleau *et al*., 2017).

$$\text{Disease Incidence} = \frac{(\text{Number of infected foliages})}{(\text{Total number of foliages})} \times 100\%$$

In order to evaluate the severity of the disease, the percentage of damaged area of foliage was calculated with the aid of a mobile application known as 'Leaf Doctor' (Pethybridge and Nelson, 2015). The disease severity was assessed using a disease rating scale proposed by Tipu *et al*. (2021), where rating scale 0 – no infection (healthy foliage); scale 1 – 1-5% of damaged leaf area; scale 2 – 6-25% of damaged leaf area; scale 3 – 26-50% of damaged leaf area; scale 4 – 51-

75% of damaged leaf area and scale 5 – 76-100% of damaged leaf area. Using the above-mentioned rating scale, the disease severity was estimated based on the following equation adopted by Chiang *et al*. (2017) as a disease severity index.

$$\text{Disease Severity Index (DSI)} = \frac{[\text{Sum (class frequency} \times \text{score rating})]}{(\text{Total number of leaves} \times \text{maximum disease rating})} \times 100\%$$

Plant growth parameters

Number of leaves per brinjal plant, plant height and number of branches per brinjal plant were counted seven weeks after transplanting.

Analysis of data

Data on disease severity and incidence were converted using the arcsine square root transformation method to stabilize variance, whereas data on the number of branches per brinjal plant and number of leaves per brinjal plant were log transformed [$\log_{10}(n)$]. The effects of the treatments on the calculated parameters were evaluated by analysis of variance (ANOVA) with the aid of Minitab version 19 statistical software. Treatment means were compared using Tukey's test at $\alpha = 0.05$.

Results and Discussion

Assessment of different treatments against leaf disease incidence and disease severity of brinjal plant

The data presented in Figure 1 illustrated that foliar spraying of garlic clove extract and salicylic acid had an effective role against leaf disease severity and disease incidence of naturally affected brinjal plant leaves at 7 WAT, whereas a significant difference ($P<0.05$) in disease severity and incidence was observed among the treatments.

According to the findings, leaf disease incidence significantly (P<0.05) declined in comparison with control when garlic clove extract and salicylic acid were applied. Besides, among these two treatments, there was no significant difference (P>0.05) obtained. Garlic clove extract and salicylic acid decreased the incidence of leaf disease by 63.6% and 88.7%, respectively, as compared to the control. In contrast, the disease incidence was statistically equivalent and significantly lower for neem leaf extract, sodium bicarbonate and eucalyptus leaf extract when compared to the control. The obtained results on disease severity illustrated that salicylic acid, sodium bicarbonate and garlic clove extract sprayings significantly (P<0.05) minimized the disease severity compared to the control, and those treatments reduced the mean disease severity by 61.4%, 92.8% and 77.1%, respectively. However, it was determined that neither eucalyptus leaf extract nor neem leaf extract significantly (P>0.05) reduced the disease severity as they were statistically similar.

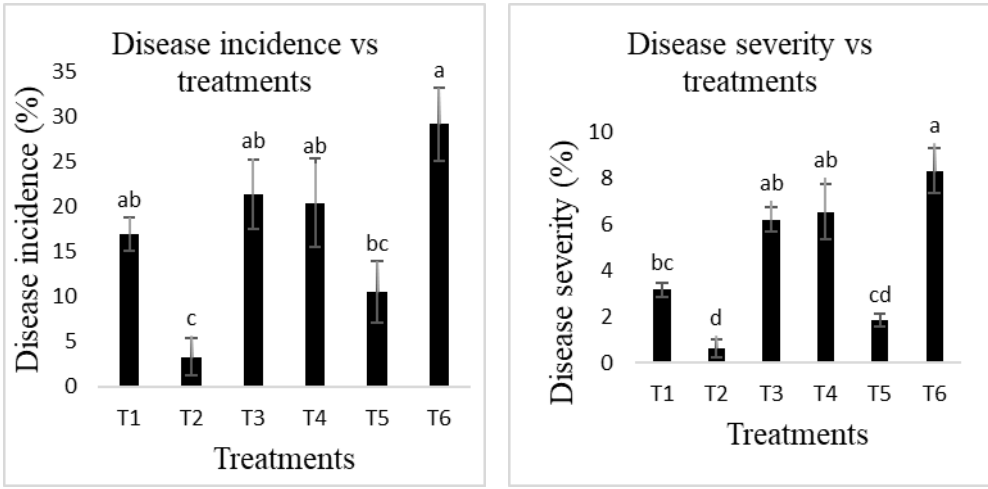

Fig. 1: Effect of different treatments against leaf disease incidence and disease severity in brinjal plant leaves. The error bar indicates the standard deviation of the mean. Values with different letters show significant differences (P<0.05).

Based on the present experiment, garlic clove extract and salicylic acid treatments considerably reduced the leaf disease severity and disease incidence

in contrast to the control. These findings of salicylic acid treatment against disease severity and incidence were concurred by Spletzer and Enyedi (1999) against Alternaria leaf spot of tomato. They discovered that spraying salicylic acid may have somewhat reduced the susceptibility of *A. solani*. When salicylic acid is injected into or sprayed onto leaves, it can stimulate the plant defense mechanism as it plays as systemic acquired resistance (SAR) inducing substance. Also, salicylic acid induces the production of pathogenesis-related proteins (PR – proteins) and acts a role in the resistance against various plant pathogens (Agrios, 2005). The derived results regarding garlic clove extract are consistent with Asaduzzaman *et al*. (2008) against Cercospora leaf spot of chilli and Nashwa and Elyousr (2012) on tomatoes against early blight, since garlic extract consists of organosulfur compounds like diallyl trisulfide, allicin and diallyl disulfide. Garlic clove extract has remarkable antifungal effects, as demonstrated by numerous studies (Martins *et al.,* 2016; Yousuf *et al*., 2010). It was also proved that sodium bicarbonate spraying was effective in reducing disease severity in brinjal leaves, and it was endorsed by El-Mougy and Abdel-Kader (2009) against early blight disease of potato. Nevertheless, this experiment did not find any significant impact of exogenous foliar spraying of sodium bicarbonate on the percentage disease incidence. Conversely, neem and eucalyptus extracts did not show effectiveness in controlling foliar diseases, contrary to the results reported by Nashwa and Elyousr (2012). The variations in the host plant factors and the ineffectiveness of these leaf extracts in controlling the diseases could be the possible reasons for this disparity.

Assessment of different treatments on growth characteristics of brinjal plant

Evaluation of different treatments on height of brinjal plant

The effects of the different treatments on the height of brinjal plant at 7 WAT are demonstrated in Table 1. Findings at 7 WAT depicted that there was a significant ($P<0.05$) influence noted in salicylic acid treatment in comparison to control. Salicylic acid treatment showed 30.8% enhancement in plant height than that of the control. The results exhibited that all the plant extracts and

sodium bicarbonate did not have the ability to significantly increase the plant height as they showed statistically (P>0.05) similar results as control.

Table 1. Effects of different treatments on the height of brinjal plant at 7 WAT

Treatments	Plant height ± SD (cm)
T_1 – Sodium bicarbonate	44.5 ± 3.4^{ab}
T_2 – Salicylic acid	51.4 ± 1.2^{a}
T_3 – Neem leaf extract	43.5 ± 2.4^{ab}
T_4 – Eucalyptus leaf extract	44.4 ± 1.6^{ab}
T_5 – Garlic clove extract	45.6 ± 0.9^{ab}
T_6 – Control	39.3 ± 3.9^{b}

Values with different letters show significant differences (P<0.05) based on Tukey test.

The data revealed that salicylic acid application offered a positive effect in terms of plant height. These consequences were in agreement with those of El-Hady *et al*. (2021) in tomato and Yildirim and Dursun (2009) in tomato who suggested that exogenous salicylic acid application exhibited significant variation in plant height when compared with control.

Evaluation of different treatments on the number of leaves per brinjal plant and number of branches per brinjal plant

Effects of GRAS compounds and control on the number of leaves per brinjal plant and number of branches per brinjal plant at 7 WAT are shown in Figure 1. The findings of the current study manifested that there was a significant (P<0.05) dissimilarity in salicylic acid treatment in comparison to control where salicylic acid application significantly increase the number of branches per brinjal plant and number of leaves per brinjal plant in its each replication when compared to control. Salicylic acid treatment increased the number of branches per plant and the number of leaves per plant by 160% and 106.5%, respectively, compared to the control. Concurrently, sodium bicarbonate (T_1), eucalyptus leaf extract (T_4), garlic clove extract (T_5) and neem leaf extract (T_3), offered no significant (P>0.05) effect in developing number of branches per brinjal plant and number of leaves per brinjal plant in comparison to control.

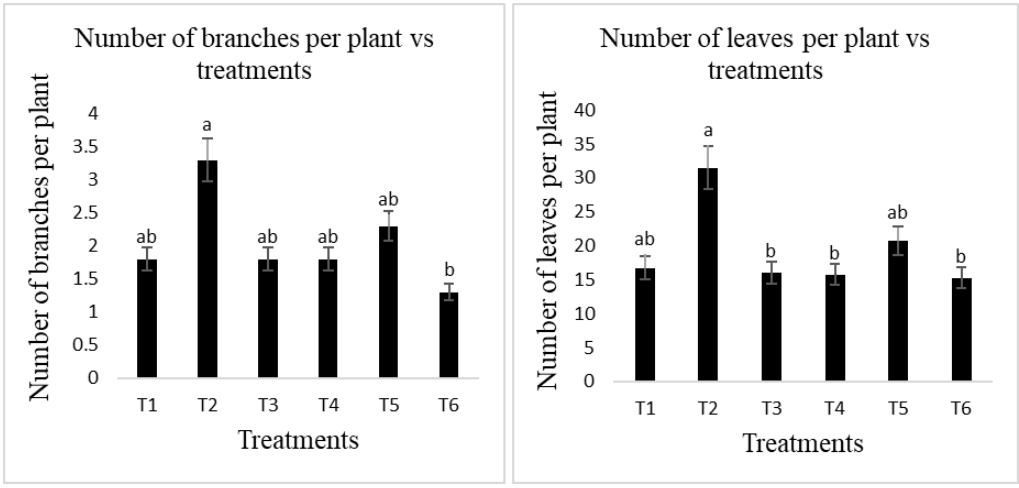

Fig. 2: Effect of different treatments on the number of leaves per brinjal plant and number of branches per brinjal plant. The error bar indicates the standard deviation of the mean. Values with different letters show significant differences ($P<0.05$).

The results of number of branches per brinjal plant and number of leaves per brinjal plant were in accordance with Gharib (2006), who reported that exogenous foliar treatment of salicylic acid showed a significant enhancement in the number of branches per plant in marjoram and basil crops when compared to no application. It is believed that salicylic acid acts in concert with other compounds, including auxin, which is known to influence cell division and expansion during shoot and root development (Larque-Saavedra, 1979; Singh and Kaur, 1980).

Conclusions

According to the overall findings, the foliar spraying of garlic clove extract and salicylic acid effectively reduced the leaf disease severity and disease incidence in brinjal plant. In the meantime, above exogenous applications were similarly proficient of minimizing leaf disease severity and disease incidence. Also, salicylic acid had a major impact in increasing the number of leaves per plant, plant height and number of branches per plant. Nevertheless, their effectiveness should be further evaluated in farmers' adoption trials and extensive field trials.

Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

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