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Research Article

APPRAISAL OF ANTIFUNGAL POTENTIAL OF CHEMICALS AND PLANT EXTRACTS AGAINST BROWN LEAF SPOT OF SOYBEAN CAUSED BY *SEPTORIA GLYCINE*

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ABSTRACT

Soybean is a legume crop originating in China. Its nutritional value, characterized by high-quality protein and unsaturated fats, makes it an excellent cholesterol-free diet option. Brown leaf spot of soybean, caused by *Septoria glycine*, is one of the most devastating diseases, resulting in significant yield losses. This study aimed to evaluate the effectiveness of new chemical fungicides (Topsin-M, Recado, Evito, Polyram-DF, and Curzate-M) at concentrations of 50, 100, and 150 ppm, and phyto-extracts (neem, clove, eucalyptus, ficus, and datura) at concentrations of 3, 5, and 7% against *S. glycine* under *in vitro* conditions. The most effective treatments from the *in vitro* tests were further investigated under greenhouse conditions using a completely randomized design with three replications per treatment. The results indicated that, among the fungicides, Topsin-M showed the lowest mycelial growth (3.5, 2.73, and 2.34 mm) at concentrations of 50, 100, and 150 ppm, respectively. Among the phyto-extracts, clove demonstrated the most effective results, with the least fungal growth (3.95 mm) at 7% concentration. Under greenhouse conditions, the best results were achieved with a combination of Topsin-M and clove, resulting in the lowest disease incidence (10.27%), followed by Topsin-M (17.65%) and clove (26.76%). This study suggests that new chemical fungicides and phyto-extracts can be effectively used as part of an integrated management strategy against brown leaf spot of soybean.

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INTRODUCTION

Pakistan spends a significant amount of foreign exchange to import edible oil and related products to meet its domestic needs. Soybean is a popular oilseed crop due to its nutritionally balanced, unique composition, and high-quality protein and oil content. The major soybean-producing countries are Brazil and Argentina (Britannica, 2022). Soybean seeds contain 40-42% protein, 20-30% carbohydrates, and 20-22% oil, along with essential vitamins and minerals (Asad et al., 2020). Isoflavones, present in soybeans, are a key component in the development of functional foods. Isoflavones exhibit significant health benefits, including anti-cancer effects, particularly in prostate and breast cancer, antioxidant properties, and the regulation of enzymatic activity in steroid metabolism and synthesis (Hu et al., 2019).

Brown leaf spot of soybean, caused by *Septoria glycines* (Hemmi), is a major limiting factor in soybean production. This disease was first reported in the United States in 1922 (Wolf and Lehman, 1924). The characteristic symptoms of brown leaf spot include yellow halos with dark brown, irregular necrotic spots on the leaves, generally appearing as early as the V2 stage of plant development (Mueller et al., 2016). Disease development due to *S. glycines* typically occurs between 15-30°C, with the optimal temperature range being 24-26°C. During the growing season, the disease spreads vertically through rain splashes, with rain before flowering promoting the development of spots on leaves (Lin et al., 2021). Fruiting bodies (pycnidia) begin to form under warm and humid conditions (Hartman et al., 2015).

Various management strategies have been employed to control Septoria brown leaf spot in soybean. The use of resistant varieties is considered the best option (Ali et al., 2016), as it poses no risks to the environment and reduces the need for chemical pesticides. However, in the absence of resistant varieties or during disease epidemics, fungicides are preferred due to their superior efficacy, ability to ensure crop quality and food safety, availability, and favorable cost-to-profit ratios (Iqbal and Mukhtar, 2020). Lin et al. (2021) demonstrated the effectiveness of chlorothalonil against brown leaf spot of soybean, with weekly applications at a dosage of 1.05 kg ha^{-1} providing effective disease control. Strobilurin fungicides have also been shown to be highly effective against *S. glycine* under *in vitro* conditions, outperforming other fungicides (Mantecón, 2008).

Concerns about the toxicity and environmental hazards of chemical fungicides, due to their long residual effects and potential harm to animal and human health, cannot be ignored (Imran et al., 2021). Moreover, with increasing awareness of health risks, there is a growing need to explore natural alternatives that are eco-friendly and non-hazardous to living organisms, including humans (Jabeen et al., 2021; Khan and Javaid, 2023; Mukhtar et al., 2023). Plant extracts have gained interest for their antifungal properties (Khanzada et al., 2016; Saeed et al., 2021; Azeem et al., 2022; Naqvi et al., 2023). Plants rich in secondary metabolites such as terpenoids, alkaloids, glycosides, flavonoids, saponins, tannins, and quinones exhibit antimicrobial activity (Ferdosi et al., 2021; Jabeen et al., 2022). The use of plant extracts in disease management has shown promising results (Iqbal et al., 2014; Javaid and Khan, 2016; Sales et al., 2016; Khan et al., 2021; Shahbaz et al., 2023). For instance, plant extract from the timber tree *Prosopis nigra* significantly reduced the severity of brown leaf spot in soybean when applied preventively (Sequín et al., 2020). Given the above rationale, this study was designed to assess the efficacy of novel fungicides and phyto-extracts against brown leaf spot disease of soybean caused by *S. glycine*.

MATERIALS AND METHODS

Isolation, purification and identification of *Septoria glycine*

Soybean leaves exhibiting typical symptoms of brown leaf spot were collected from the Agronomy Farm Area at the University of Agriculture, Faisalabad. These leaf samples were transported to the Mycology Laboratory in brown paper bags. The samples were washed with tap water to remove dust and then cut into small pieces (3-5 mm) ensuring that both healthy and diseased portions were included. The cut pieces were surface-sterilized by immersion in 1% sodium hypochlorite (Clorox) for 30 sec, followed by three rinses with distilled water to remove any residual sterilant.

Autoclaved potato dextrose agar (PDA) medium was poured into sterilized Petri plates. After the medium solidified, the dried leaf pieces were placed onto the Petri plates. The plates were sealed with parafilm tape and incubated at 25 ± 2°C. Once mycelial growth began, it was purified using the hyphal tip culture method (Leyronas et al., 2012). Within seven days of incubation, a purified culture was obtained and examined under a microscope for morphological identification. The mycelia displayed scanty growth on PDA, with cottony white, irregularly branched, and septate hyphae. The spores of *S. glycines* were straight to curved, filiform, hyaline, with 2-4 septations, and had rounded to pointed ends (Rawal and Sohi, 1983).

Confirmation of pathogenicity

Pathogenicity was confirmed using the leaf detachment method. Soybean seeds were grown in plastic pots (10 × 13 cm) containing peat moss under controlled conditions. Young leaves, 40 days old, were collected, washed with tap water, surface sterilized with sodium hypochlorite, and placed on sterilized moist filter paper in a clean tray. Mycelial plugs (6 mm in diameter) from the pure culture of *S. glycines* were obtained using a cork borer and placed on each leaf, except for the control. The tray was then sealed with parafilm tape and incubated at 25°C with a 12-hour photoperiod. Browning symptoms began to appear four days after inoculation, and the pathogen was re-isolated from these leaves and compared with the parent culture for confirmation.

In-vitro management of _S. glycine_ using chemicals

Five chemical fungicides (Topsin-M, Recado Super, Polyram DF, Evito, and Curzate-M) along with a control treatment were tested against *S. glycine* using the poisoned food technique at three different concentrations: 50, 100, and 150 ppm. The stock solutions for each fungicide were prepared by dividing the percentage of active ingredient by 100 and adding that quantity to 100 ml of distilled water. Then, 0.5, 1.0, and 1.5 ml of the stock solution were added to 100 ml of PDA media to achieve concentrations of 50, 100, and 150 ppm, respectively. The amended media was poured into Petri plates, and a 6 mm diameter mycelial plug was placed at the center of the solidified media. The experiment was conducted using a Completely Randomized Design (CRD) with three replications for each treatment. Fungal growth was measured daily for four consecutive days at 24-hour intervals.

In vitro management of _S. glycine_ using plant extracts

Five natural plant extracts (neem, clove, datura, ficus, and eucalyptus) were evaluated against *S. glycine in vitro* using the poisoned food technique at three concentrations: 3%, 5%, and 7%, by adding the required aqueous solutions to 100 ml of distilled water. The preparation of concentrations and the inoculation of Petri plates were conducted following the procedure described above.

Management of brown leaf spot of soybean under greenhouse conditions

Soybean plants were grown in earthen pots (15.24 × 10.16 cm) in the greenhouse of the Department of Plant Pathology, UAF. The inoculum was prepared by adjusting the concentration of conidial spores to 4×10^3 - 5×10^4 cfu per ml using a Neubauer hemocytometer (Sequín et al., 2020) and sprayed on plants at the V2 stage. The most effective chemical (Topsin-M) and plant extract (clove) identified under laboratory conditions were further investigated individually and in combination under greenhouse conditions against brown leaf spot of soybean. Two concentrations (0.5% and 1%) were prepared for the greenhouse experiment by mixing the most effective concentrations of both treatments: 150 ppm of Topsin-M and 7% clove extract. Each treatment had three replications, with each replication containing three soybean plants. The control group was left untreated. After the first symptoms appeared in the control group, disease incidence (%) was recorded twice at 7-day intervals. The brown spot diagrammatic scale, with representative leaf pictures, was used to measure disease incidence (Cruz et al., 2010).

Statistical analysis

The laboratory experiments included 16 treatments for both fungicides and plant extracts, while the greenhouse experiments included seven treatments. CRD was used for the *in vitro* experiments, and a Randomized Complete Block Design (RCBD) was employed for the greenhouse experiments. Data were subjected to analysis of variance (ANOVA) at a 0.05% level of significance. Fisher's least significant difference (LSD) test was used to separate the means at $p \leq 0.05$. Statistical analysis was performed using the "Statistics 8.1" software.

RESULTS

Evaluation of synthetic chemicals against _S. glycine_ under _In vitro_ conditions

Among all the treatments, Topsin-M showed the least fungal growth, measuring 2.85 mm, followed by Recado (5.83 mm), Evito (7.79 mm), Curzate-M (10.32 mm), and Polyram-DF (11.62 mm) (Figure 1A). The interaction between treatments and concentrations revealed that Topsin-M exhibited minimal fungal growth at 150, 100, and 50 ppm, with measurements of 2.34 mm, 2.73 mm, and 3.50 mm, respectively. This was followed by Recado (6.74 mm, 5.70 mm, 5.04 mm), Evito (9.93 mm, 7.27 mm, 2.57 mm), Curzate-M (9.67 mm, 10.25 mm, 11.04 mm), and Polyram-DF (11.25 mm, 11.50 mm, 12.12 mm) (Figure 1B). The impact of the interaction between treatments and duration showed that Polyram-DF resulted in the maximum mycelial growth, with measurements of 7.72 mm, 9.11 mm, 12.05 mm, and 17.61 mm after 24, 46, 72, and 96 h, respectively. In contrast, Topsin-M exhibited the minimum mycelial growth, with values of 2.44 mm, 2.65 mm, 3.00 mm, and 3.33 mm after the same durations, followed by Recado (5.11 mm, 5.20 mm, 5.73 mm, 7.28 mm), Evito (5.77 mm, 6.70 mm, 8.42 mm, 10.25 mm), and Curzate-M (6.32 mm, 7.15 mm, 10.82 mm, 16.98 mm) (Figure 1C).

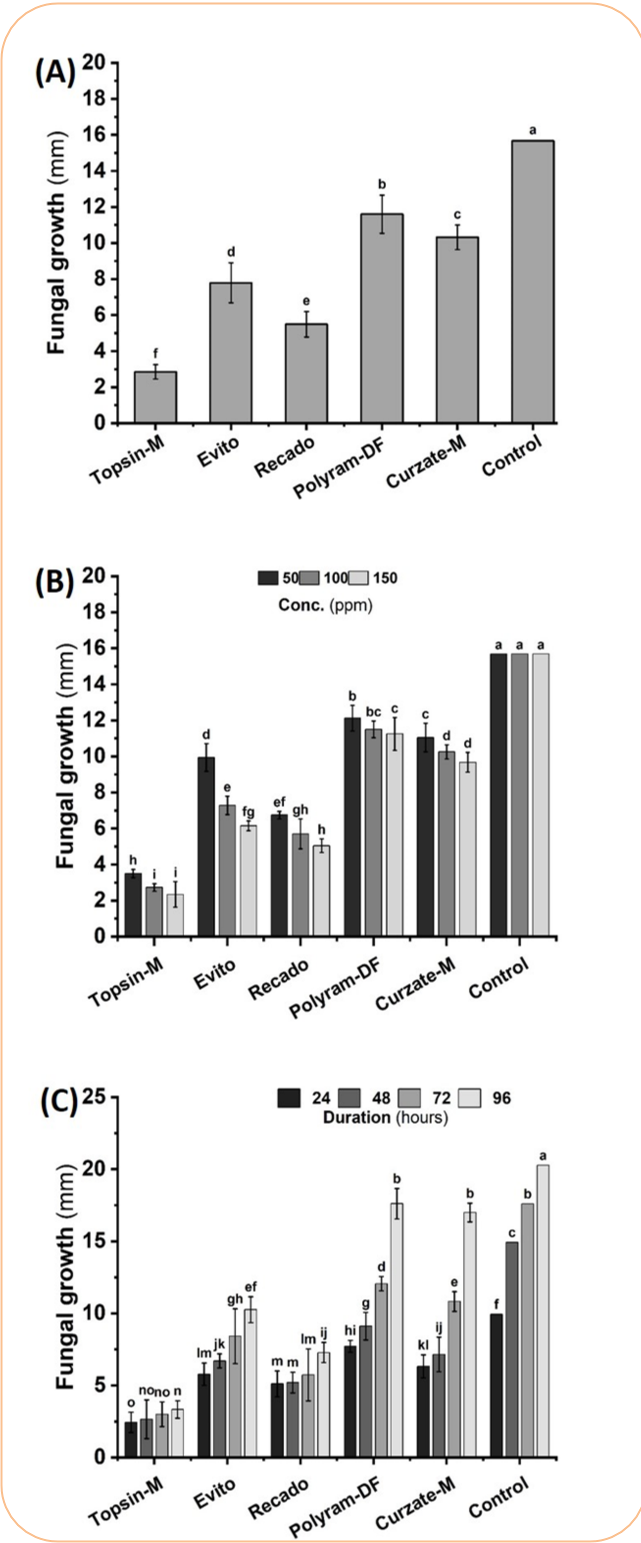

Figure 1. (A) the main impact of different fungicides on mean mycelial growth of *S. glycine*, (B) the impact of fungicides with effect of concentrations on growth of *S. glycine* and (C) the impact of fungicides with effect of time durations on mycelial growth of *S. glycine* under *in vitro* environment.

Evaluation of phyto-extracts against *S. glycine* under *in vitro* conditions

Among all the phyto-extracts tested, clove exhibited the least fungal growth (4.25 mm), followed by datura (6.20 mm), ficus (8.65 mm), neem (9.78 mm), and eucalyptus (10.58 mm), as compared to the control (Figure 2A). The interaction between treatments and concentrations revealed that clove consistently resulted in the minimum fungal growth at 7%, 5%, and 3% concentrations (3.95 mm, 4.12 mm, and 4.68 mm, respectively). This was followed by datura (5.79 mm, 6.12 mm, 6.70 mm), ficus (4.29 mm, 8.11 mm, 13.55 mm), neem (8.13 mm, 11.34 mm, 9.88 mm), and eucalyptus (9.04 mm, 10.39 mm, 12.30 mm) at the same concentrations (Figure 2B).

The effect of phyto-extracts over different time durations indicated that eucalyptus extract was the least effective, showing maximum mycelial growth (3.02 mm, 9.32 mm, 13.01 mm, 16.96 mm) after 24, 48, 72, and 96 h, respectively. In contrast, clove was the most effective, resulting in the lowest fungal growth. The interaction between treatment and time (T×D) showed that clove maintained minimal fungal growth across all time points (3.69 mm, 3.85 mm, 4.53 mm, and 4.92 mm). Other treatments demonstrated moderate effects, such as datura (5.68 mm, 5.92 mm, 6.19 mm, 7.03 mm), ficus (2.07 mm, 6.55 mm, 10.25 mm, 15.74 mm), and neem (4.09 mm, 8.17 mm, 13.05 mm, 16.96 mm) (Figure 2C).

Management of brown leaf spot of soybean under greenhouse conditions

All treatments demonstrated a significant controlling effect against brown leaf spot of soybean. Among the combinations tested, Topsin-M + Clove proved to be the most effective, resulting in the lowest disease incidence of 10.27%, followed by Topsin-M (17.65%), and Clove (26.76%) (Figure 3A). Each treatment was applied at two concentrations. The interaction between treatments and concentrations revealed that at 0.5% and 1% concentrations, the Topsin-M + Clove combination exhibited the lowest disease incidence of 14.97% and 5.57%, respectively, followed by Topsin-M alone (23.03% and 12.27%), and Clove (33.89% and 19.62%) (Figure 3B). Moreover, the interaction between treatment and application days showed that the Topsin-M + Clove combination resulted in minimal disease incidence of 12.08% and 8.46% after 7 and 14 days of application, respectively. In contrast, the solo application of Clove provided the least effective control, with the highest disease incidence of 28.66% and 24.85% over the same periods (Figure 3C).

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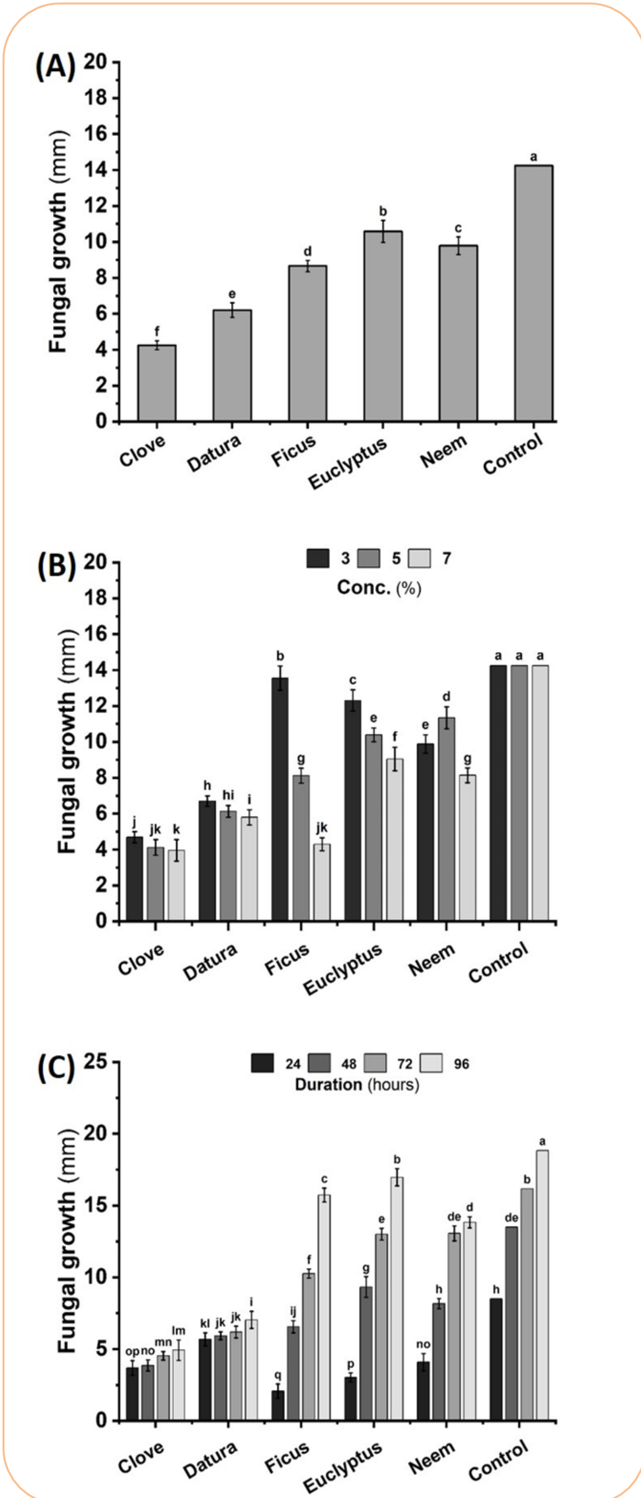

Figure 2. (A) the mean mycelial growth of *S. glycine* after phytochemical treatments, (B) the impact of phytochemicals in relation with concentrations on growth of *S. glycine* and (C) effect of treatments in relation with time durations on mycelia growth of *S. glycine* under laboratory conditions.

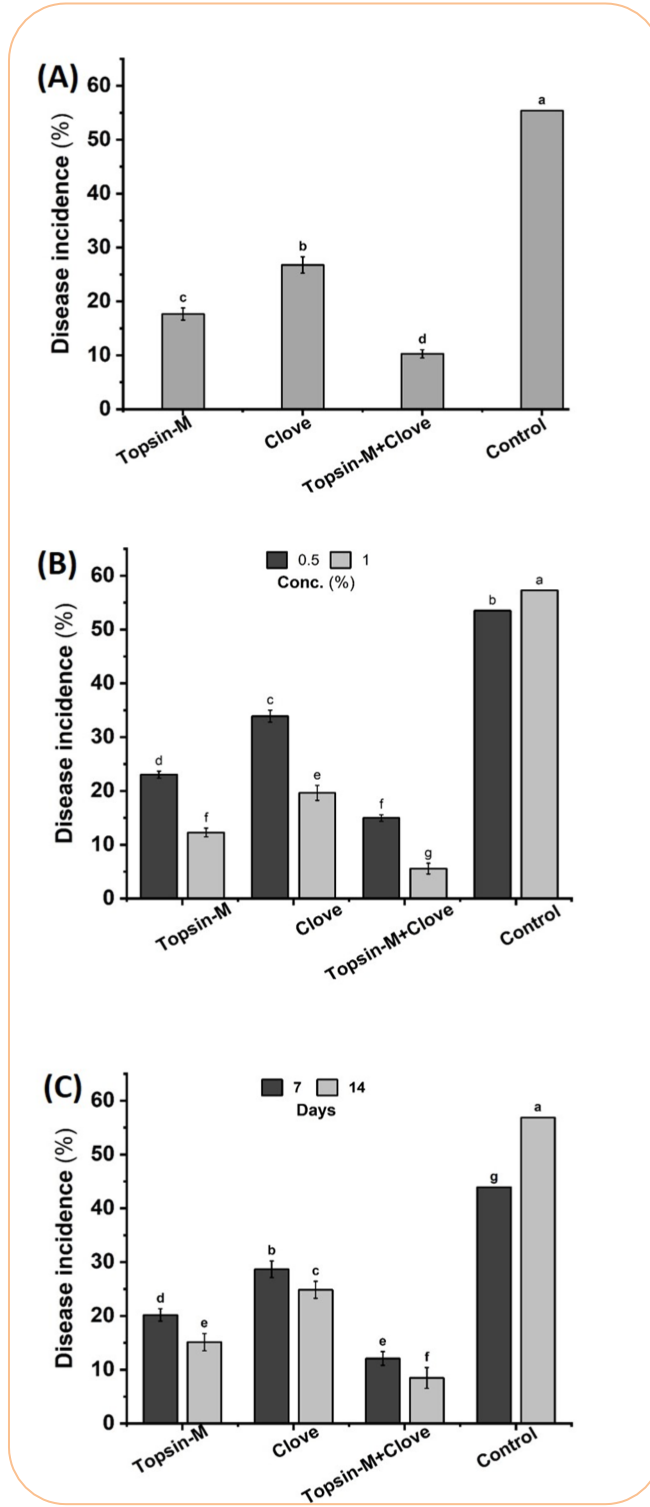

Figure 3. (A) Mean disease incidence of Brown leaf spot of soybean after application of most effective treatments, (B) the impact of treatments with effect to concentrations against Brown leaf spot of soybean and (C) the mean disease incidence after application of treatments with effect to days.

DISCUSSION

Among the various management strategies used to combat plant diseases, chemical pesticides are widely favored due to their long-lasting effects, easy availability, and lower cost. In this study, five synthetic chemicals were evaluated for their effectiveness in controlling *S. glycine*. These included Topsin-M (70% WP), Recado Super (32% SC), Evito (18+25%), Polyram-DF (70%), and Curzate-M (72% WP).

Among these new-generation chemical pesticides, Topsin-M produced the best results, inhibiting the growth of *S. glycine* under both *in vitro* and greenhouse conditions. Topsin-M contains thiophanate-methyl, which belongs to FRAC Group 1 (Benzimidazoles). This chemical acts as a tubulin inhibitor, preventing microtubule assembly within cells. It exhibits preventive, systemic, and curative effects, which are tailored to specific crops, fungi, and environmental conditions (Loughlin, 2017).

Another chemical that significantly reduced the growth of *S. glycine* was Recado Super, a mixture of azoxystrobin (200 g/L) and difenoconazole (120 g/L). Azoxystrobin is particularly effective against fungal conidial germination, while difenoconazole is more potent against mycelial development of fungi (Wang et al., 2016). The metabolic mechanism of azoxystrobin is similar to that of naturally occurring strobilurins, offering a broad spectrum of action against fungi from the Oomycetes, Ascomycetes, Deuteromycetes, and Basidiomycetes taxonomic groups. Azoxystrobin functions by binding to the cytochrome bc1 complex in the fungal mitochondria, disrupting electron transport and oxidative phosphorylation, the processes by which energy is generated (Joseph, 1999).

On the other hand, difenoconazole acts as a sterol demethylation inhibitor, preventing fungal growth by blocking the biosynthesis of ergosterol in cell membranes. This reduction in sterol production hinders further infection and invasion of host tissues by slowing or stopping fungal growth.

These results are consistent with the findings of Mantecón (2008), who evaluated four conventional chemical pesticides. The best results in reducing disease incidence and severity were observed with strobilurin fungicides, specifically pyraclostrobin plus epoxiconazole, trifloxystrobin plus cyproconazole, and azoxystrobin plus cyproconazole. This study is further supported by Cruz et al. (2010), who discussed the effectiveness of triazole and strobilurin-based fungicides against *S. glycine*. Similarly, de Oliveira et al. (2000) found azoxystrobin, followed by difenoconazole and benomyl, to be the most effective fungicides against *S. glycine*. Furthermore, Batzer et al. (2016) highlighted the potential of fluopyram fungicide as a seed treatment against brown leaf spot of soybean.

Chemical pesticides play a crucial role in modern agriculture, helping to meet the demands of a growing population. However, their highly toxic nature poses significant risks to both the environment and human health when used extensively and without proper safety measures. Additionally, they negatively impact nearby organisms, including animals and agricultural workers, who are exposed to these pollutants both directly and indirectly (Rani et al., 2021).

To address these concerns, plant extracts have been explored as alternative methods for disease management. Plant extracts are eco-friendly, less toxic, and possess antifungal properties, making them biodegradable and safe to use (Shuping and Eloff, 2017). In this study, five plant extracts (Datura, Clove, Ficus, Eucalyptus, and Neem) were evaluated against *S. glycine* under *in vitro* conditions. Among these, Clove was found to be the most effective.

Clove contains eugenol, acetyleugenol, chavicol, acetyl salicylate, and humulenes (Zheng et al., 1992), which exhibit broad-spectrum antifungal activity. Clove oil and eugenol significantly inhibited the production of germ tubes, a key factor in the pathogenicity of various fungi. Moreover, clove extract contains azole compounds that disrupt fungal cell development by interfering with sterol biosynthesis, reducing ergosterol production, which is essential for maintaining cell membrane integrity (Pinto et al., 2009).

The effectiveness of Clove extract was further demonstrated when combined with the chemical Topsin-M in greenhouse experiments. This combination showed significant control over *S. glycine* with the lowest disease incidence.

Our findings are supported by Sequin et al. (2020), who investigated the antifungal activity of the timber tree *P. nigra* extract, both alone and in combination with synthetic chemicals. The methanol leaf extract of *P. nigra*, rich in the antifungal component tryptamine, exhibited the strongest activity against *S. glycines*, enhancing the effectiveness of difenoconazole when used together. Similarly, Lal et al. (2016) found that neem extract was the most effective biocontrol agent among five tested plant extracts against *S. lycopersici*, followed by ginger, garlic, onion, and mustard. Previous studies have also highlighted the antifungal efficacy of neem and Eucalyptus against various fungal diseases (Khan and Javaid, 2021; Javaid et al., 2022).

CONCLUSION

The findings of the present study indicate that both synthetic fungicides and phytoextracts can be used effectively to manage brown leaf spot of soybean, caused by *Septoria glycine*. Among the phytoextracts tested, clove demonstrated particularly promising results against *S. glycine*, suggesting its potential as an alternative for managing brown leaf spot. For sudden outbreaks of the disease, synthetic chemicals like Topsin-M can be an effective solution. Additionally, many plants possess antimicrobial properties; identifying and utilizing these plant-based ingredients could become a valuable tool in the bio-management of plant diseases.

AUTHORS' CONTRIBUTIONS

FM executed the field research and wrote manuscript; NAR and MA conceived the idea and supervised the work; MAl and KN reviewed and edited the manuscript; GAK and MUA helped in statistical analysis of the data; whereas FA and MH helped in conducting experiments and writing the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERNCES

Ali, S., Anjum, S., Zeshan, M.A. Arshad, Ahmed, M.N., Ghani, M.U., 2016. Evaluation of nutritional amendments, plant extracts and chemicals for the management of stem rot of rice. Pakistan Journal of Phytopathology 28, 45-55.

Asad, S.A., Wahid, M.A., Shaheen, F., Raza, A., Farooq, M., 2020. Soybean production in Pakistan: Experiences, challenges and prospects. International Journal of Agriculture and Biology 24, 995-1005.

Azeem, W., Mukhtar, T., Hamid, T., 2021. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in tomato. Pakistan Journal of Zoology 53(1), 119-125.

Batzer, J.C., Kandel, Y.R., Bradley, C.A., Chilvers, M.I., Tenuta, A.U., Wise, K.A., Hernández, E., Mueller, D.S., 2016. Effect of seed treatment on early season brown spot caused by *Septoria glycines* of soybean. Plant Health Progress 17(4), 223-228.

Britannica, 2022. Soybean, Description, Cultivation, Products, & Facts. Available online with updates at https://www.britannica.com/plant/soybean

Cruz, C.D., Mills, D., Paul, P.A., Dorrance, A.E., 2010. Impact of brown spot caused by *Septoria glycines* on soybean in Ohio. Plant Disease 94(7), 820-826.

de Oliveira, W.F., Caetano, F.V., Dias, E.M., Batista, R.G., Nonato, A.R., 2000. Efficiency of fungicides in the control of *Septoria glycines* and *Cercospora kikuchii* on soybean (*Glycine max* L.). Pesquisa Agropecuária Tropical 30(2), 63.

Ferdosi, M.F.H., Javaid, A., Khan, I.H., Ahmad, S., Shad, N., 2021. Analysis of n-butanol flower extract of *Cassia fistula* through GC-MS and identification of antimicrobial compounds. Pakistan Journal of Phytopathology 33, 103-107.

Hartman, G., Rupe, J., Sikora, E., Domier, L., Davis, J., Steffey, K., 2015. Compendium of Soybean Diseases and Pests, Fifth Edition. American Phytopathological Society, St. Paul, Minnesota.

Hu, C., Wong, W.T., Wu, R., Lai, W.F., 2019. Biochemistry and use of soybean isoflavones in functional food development. Critical Reviews in Food Science and Nutrition 60, 2098-2112.

Imran, M., Ali, E.F., Hassan, S., Abo-Elyousr, K.A.M., Sallam, N.M., Khan, M.M.M., Younas, M.W., 2021. Characterization and sensitivity of *Botrytis cinerea* to benzimidazole and succinate dehydrogenase inhibitors fungicides, and illustration of the resistance profile. Australasian Plant Pathology 50, 589-601.

Iqbal, U., Mukhtar, T., 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52(2), 709-715.

Iqbal, U., Mukhtar, T., Iqbal, S.M., 2014. *In vitro* and *in vivo* evaluation of antifungal activities of some antagonistic plants against charcoal rot causing fungus, *Macrophomina phaseolina*. Pakistan Journal of Agricultural Sciences 51 (3), 689-694.

Jabeen, N., Javaid, A., Shoaib, A., Khan, I.H., 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel*.

Journal of Plant Pathology 103, 901-913.

Jabeen, N., Khan I.H., Javaid, A., 2022. Fungicidal potential of leaf extract of *Datura metel* L. to control *Sclerotium rolfsii* Sacc. Allelopathy Journal 56, 59-68.

Javaid, A., Amin, M., Chaudhury, F.A., Khan, I.H., Jabeen, N., 2022. Identification of antifungal compounds from leaf extract of *Eucalyptus citriodora* against *Ascochyta rabiei*. Pakistan Journal of Science 74, 243-249.

Javaid, A., Khan, I.H., 2016. Management of collar rot disease of chickpea by extracts and soil amendment with dry leaf biomass of *Melia azedarach* L. Philippine Agricultural Scientist 99, 150-155.

Joseph, R.S.I., 1999. Metabolism of Azoxystrobin in plants and animals. Special Publication-Royal Society Of Chemistry 265-278.

Khan, I.H., Javaid, A., 2021. Identification of biologically important compounds in neem leaves through GC-MS analysis. Jordan Journal of Pharmaceutical Sciences 14, 359-366.

Khan, I.H., Javaid, A., 2023. Antifungal potential of *Chenopodium quinoa* root extract against *Macrophomina phaseolina* (Tassi) Goid. Allelopathy Journal 58(1).

Khan, I.H., Javaid, A., Naqvi, S.F., 2021. Molecular characterization of *Penicillium expansum* isolated from grapes and its management by leaf extract of *Chenopodium murale*. International Journal of Phytopathology 10(1), 29-35.

Khanzada, M.A., Tanveer, M., Maitlo, S.A., Hajano, J., Ujjan, A.A., Syed, R.N., Lodhi, A.M., A. Rajput, Q., 2016. Comparative efficacy of chemical fungicides, plant extracts and bio- control agents against *Fusarium Solani* under laboratory conditions. Pakistan Journal of Phytopathology 28, 45-55.

Lal, M., Dabbas, M.R., Singh, S.K., Kumar, S., Kumar, N., 2016. Efficacy of various bio-agents and plant extract against *Septoria lycopersici*. International Journal of Plant Protection 9, 177-182.

Leyronas, C., Duffaud, M., Nicot, P.C., 2012. Compared efficiency of the isolation methods for *Botrytis cinerea*. Mycology 3, 221-225.

Lin, H.A., Villamil, M.B., Mideros, S.X., 2021. Characterization of Septoria brown spot disease development and yield effects on soybean in Illinois. Canadian Journal of Plant Pathology 43, 62-72.

Loughlin, N.O., 2017. Office of Chemical Safety and Pollution Prevention. United States Environmental Protection Agency Washington, DC. 20460.

Mantecón, J.D., 2008. Efficacy of chemical and biological strategies for controlling the soybean brown spot (*Septoria glycines*). Ciencia e Investigación Agraria 35, 211-214.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., Robertson, A., 2016. In: Foliar Diseases. A Farmer's Guid to Soybean Diseases pp. 37-74.

Mukhtar, T., Vagelas, I., Javaid, A., 2023. Editorial: new trends in integrated plant disease management. Frontiers in Agronomy. 4:1104122. doi: 10.3389/fagro.2022.11041221

Naqvi, S.F., Javaid, A., Khan, I.H., 2023. Fungicidal activity of stem extract of *Chenopodium murale* L. against pathogen of Fusarium wilt of tomato. Allelopathy Journal 59(1).

Pinto, E., Vale-Silva, L., Cavaleiro, C., Salgueiro, L., 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and Dermatophyte species. Journal of Medical Microbiology 58, 1454-1462.

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A.S., Srivastav, A.L., Kaushal, J., 2021. An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production 283, 124657.

Rawal, R.D., Sohi, H.S., 1983. Morphological and cultural studies of *Septoria vignicola*. Indian Institute of Horticultural Research 19, 83-89.

Saeed, M., Mukhtar, T., Khan, M.A., 2021. Assessment of nematicidal potential of *Cannabis sativa* and *Azadirachta indica* in the management of root-knot nematode (*Meloidogyne javanica*) on peach. Pakistan Journal of Agricultural Sciences, 58(5), 1555-1561.

Sales, M.D.C., Costa, H.B., Fernandes, P.M.B., Ventura, J.A., Meira, D.D., 2016. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pacific Journal of Tropical Biomedicine 6, 26-31.

Sequín, C.J., Sampietro, D.A., Sgariglia, M.A., Soberón, J.R., Catalán, C.A.N., Aceñolaza, P.G., 2020. Use of extracts from Prosopis nigra in the control of Cercospora kikuchii and Septoria glycines. Indian Crops Production 158, 112979.

Shahbaz, M., Akram, A., Raja, N.I., Mukhtar, T., Mehak, A., Fatima, N., Ajmal, M., Ali, K., Mustafa, N., Abasi, F. 2023. Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease. PLoS ONE 18(2): e0274679. https://doi.org/10.1371/journal.pone.0274679

Shuping, D.S.S., Eloff, J.N., 2017. The use of plants to protect plants and food against fungal pathogens: a Review. African Journal of Traditional, Complementary and Alternative Medicines 14, 120-127.

Wang, H., Huang, Y., Wang, J., Chen, X., Wei, K., Wang, M., Shang, S., 2016. Activities of azoxystrobin and difenoconazole against Alternaria alternata and their control efficacy. Crop Protection 90, 54-58.

Wolf, F.A., Lehman, S.G., 1924. Report of Division of Plant Pathology. Forty-Seventh Annual Report of North Carolina Agricultre Expert Statistic Fiscal Year 83-85.

Zheng, G.Q., Kenney, P.M., Lam, L.K.T., 1992. Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. Journal of Nature Products 55, 999-1003.