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# Integrated Biological Control of *Eichhornia crassipes* (Mart.) Solms Using Phytopathogenic Fungi and Water Hyacinth Mites: *Orthogalumna terebrantis*

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**ABSTRACT**

*Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth, is considered one of the most noxious aquatic invasive plants, which is also listed under the "hundred of the world's worst invasive alien species" list. The present study was based on the isolation and identification of phytopathogenic fungi as potential biological control agents for water hyacinth and to determine integration along with water hyacinth mites (Orthogalumna terebrantis) as an effective biological control method. Six fungal species were identified as effective biological control agents from 28 total fungal isolates. Potential fungal species were identified as Trichoderma* sp.*, Penicillium* sp.*, Exserohilum* sp.*, Alternaria* sp.*, Fusarium* sp.*, and Aspergillus* sp. *using microscopic features. The greatest level of pathogenicity (73.8±7.3%) was observed in Alternaria* sp.*, with descending levels of pathogenicity noted in Fusarium* sp. *(57.5±4.8%), Exserohilum* sp. *(56.4±2.7%), Aspergillus* sp. *(45.6±1.7%), Penicillium sp. (36.5±3.7%), and Trichoderma* sp. *(33.4±1.4%) after 28 days. Damage due to water hyacinth mites was recorded as 11.6±0.7% of the total leaf area after four weeks of introduction of 50 mites per plant. The results obtained from the integrated effect study revealed that the pathogenicity of identified phytopathogenic fungal species significantly increased ($p<0.05$) when introduced with water hyacinth mites. Fusarium* sp. *has increased its pathogenicity from 73.8±7.3% to 92.8 ±7.6% of the total leaf. The pathogenicity of the remaining two fungal species, namely Alternaria* sp. *and Exserohilum* sp.*, exhibited significant impact, ranging from 57.5±4.8% to 78.5±5.2% and from 56.4±2.7% to 75.3±4.8% of the entire leaf area, respectively. The study findings indicated that combining water hyacinth mites with phytopathogenic fungi is more effective than employing individual pathogenic fungal agents in isolation. Thus, the present study provides baseline data for future research to use fungi as a potential biological control method for water hyacinth.*

**KEYWORDS:** *Water hyacinth, Water hyacinth mite, Fusarium* sp.*, Alternaria* sp.*, Exserohilum* sp.*, Biological control*

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## 1 INTRODUCTION

Invasive aquatic plants spread rapidly and invaded many countries (Wilson et al. 2005; Riis et al. 2012) while creating several problems in freshwater biodiversity following the destruction of species richness (Coetzee et al. 2009) and affecting the economy and recreational use of the freshwaters (Coetzee et al. 2009).

In Sri Lanka and other tropical and temperate countries, the water hyacinth, Eichhornia crassipes (Japan Jabara), is regarded as one of the worst aquatic weeds (Coetzee et al. 2009; Ekanayake et al. 2017; Ekanayake et al. 2021). Water hyacinth is capable of growing within different types of habitats, including rivers, lakes, wetlands, etc. It typically favours nutrient-rich waters, but it can endure a variety of nutrients (Hill & Day 1998), temperature, and pH levels (Wilson et al. 2005). The water hyacinth typically grows in a wide range of temperatures, from 1 to 40°C (optimum growth at 25–27.5 0C), and the optimal pH is 6–8. (Wilson et al. 2005)

Annual recurrent costs associated with water hyacinth are estimated to be over $ 100 million worldwide (Hill & Day 1998). Water hyacinth's impact on water quality has primarily been studied in relation to the effects of dense mats produced by the intertwining of individual plants. (Rommens et al. 2003). The most commonly documented effects on water quality include lower phytoplankton productivity and dissolved oxygen (Perna & Burrows 2005; Villamagna & Murphy 2010).

Moreover, it creates several problems for many human uses in socio-economic aspects such as boating access, navigability, and recreation, power generation plants &, etc (Villamagna & Murphy 2010). Water hyacinth can exceed open-water evaporation rates by a factor of 10 in some areas, so it will be a serious problem where water resources are limited (Villamagna & Murphy 2010). Invasion of water hyacinth has negative impacts on human health as it provides breeding grounds for disease-causing mosquito vectors (Patel 2012).

Adaptability of these plants to the least-competed ecological conditions makes them difficult to control and difficult to eradicate (Gutierrez et al. 1996). Control methods include physical methods, chemical methods, and biological methods (Villamagna & Murphy, 2010). Usually, mechanical removal methods are used to remove water hyacinth, and this is not a long-term solution for the problem, as reinfestation occurs within a very short period of time (Patel 2012). However, each of these methods has its advantages and disadvantages (Patel 2012; Liyanage & Manage 2016b). Therefore, selection of a control method must be based on site-specific conditions, including size and spatial configuration of the area to be controlled, seasonal weather patterns, designated uses of the water body, and budget constraints (Gibbons et al. 1994).

For severe water hyacinth infestations, biological control has been favoured because it is affordable, sustainable, and has no adverse effects on the environment (Centre 1994; Julien

2001). A variety of biological agents, such as fungi and arthropods, have been employed globally to manage water hyacinth in water bodies (Patel 2012). Seven arthropod biocontrol agents, including the weevils *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache, were employed in South Africa to handle this weed (Morris et al. 1999). In some regions of the globe, including Lake Victoria, where the introduction of the two weevils, *N. eichhorniae* and *N. bruchi*, reduced the weed infestation from 20,000 to 2,000 ha in a period of five years, water hyacinth has been completely controlled. (Villamagna & Murphy, 2010).

Therefore, the aloe effects of both arthropods and phytopathogenic fungi can be enhanced if those two biological control agents are integrated (Jimenez & Balandra, 2007). A species of weevil, *N. eichhorniae* was introduced to Sri Lanka as a biological control agent in 1988 to control the propagation of water hyacinth. However, it was not effective in controlling the weed (EFL 2015).

The infestation of water hyacinths in Sri Lanka had several negative effects on the country's economy and natural ecosystems because of its high tendency to accumulate more sediment. This causes wetlands to transition into terrestrial environments and results in increased water loss due to increased transpiration. The result of such uniform stands of alien invasive plants is a decrease in native biological diversity across the country (Bambaradeniya 2002). Department of Irrigation shows that the majority of irrigational zones of the countries are invaded by water hyacinth. Water hyacinth has affected the irrigation schemes in Kurunegala, Puttalam, Colombo, Ampara, Mahanuwara, Badulla, Anuradhapura, etc. The continuous spreading of water hyacinth increases the maintenance cost of irrigational schemes and directly affects the economy of the country (Ministry of Irrigation & Water Resources Management 2017). Approximately more than 45% of the irrigation systems were invaded by invasive aquatic plants, and each year the Department of Irrigation in Sri Lanka has to spend at least 323.5 million rupees for the management of the aquatic invasive plant species (Irrigation Department 2017). Thus, there are very limited studies on the integration of two biological control agents for the effective management of water hyacinth proliferation. Hence, the present study was undertaken to find out the fungi species associated with water hyacinth as a potential biological control agent and its effective management using integration with the water hyacinth mite

## 2 METHODOLOGY

### 2.1 Sample collection

Water hyacinth plants with fungal disease symptoms were collected from Bellanwila Wetland Park, and healthy water hyacinth plants that were not affected by pathogens, arthropods, or other pests were collected from the Gampaha area and transferred into UV-sterilised sealed plastic bags. Triplicate water samples were collected from each location into sterilised 1L plastic bottles, where sediment samples were collected from 0-3cm depth at

each location and transferred into UV-sterilised black plastic bags. Collected samples were placed in an ice box and transported to the laboratory within 4 hours, and stored at 4ºC until analysis.

**2.2 Isolation and identification of water hyacinth mite**

Water hyacinth plants from each sampling site were collected and observed. Parasitic mites located on the leaves were isolated and observed using microscopy, originating from the leaves that exhibited damage. Mites were collected using a fine brush and reared under laboratory conditions, providing undamaged water hyacinth plants as a food source.

**2.3 Measurement of damage caused to the plant by the water hyacinth mite**

Healthy water hyacinth plants were maintained as stock cultures in large cement tanks under natural sunlight. Aerated tap water was used as the medium without supplementation of nutrients. Tanks were maintained properly under natural sunlight throughout the study period.

Six free-floating healthy plants of more or less the same size were selected for the exposure study. The mean surface area of the plant leaves was calculated, and 50 water hyacinth mites were introduced into each plant, and the whole tank accounted for a total number of 300 mites at the beginning. Tanks were covered separately by a fine net (and kept under natural sunlight, without adding nutrients. Visual observations were taken by measuring the holes of feeding galleries made by mites. Initial surface area and the damaged surface area of the leaves of water hyacinth plants were recorded after four weeks, following Ray & Hill 2013. The control setup was prepared, providing the same conditions and maintained without introducing mites.

**2.4 Isolation of fungi from invasive plants**

Fungal-infected plant parts were washed three times in running water. Small pieces (1-2 cm) [ leaves and petioles] of the margins of lesions were cut and surface sterilised by sequential immersion in 70% ethanol, 10% hypochlorite, and finally with sterilised water (Jimenez and Balandra 2007). Then, plant parts were placed on 1.5% Potato Dextrose Agar (PDA) plates. Before the experiment, the PDA medium was changed with the addition of an antibiotic (tetracycline) in order to inhibit undesirable bacterial contaminations, sealed by parafilm, and incubated at 28°C for 2 to 3days until fungal colonies appeared (Conway 1976).

**2.5 Isolation of fungi from water and sediment samples**

A sterile 0.9% saline solution was used to dissolve 10 ml of water samples and 10 g of soil samples that were taken from the location. Tenfold serial dilution was made for soil and water samples, and the pour plate method was used to isolate fungi using PDA medium. After three days of incubation at 28°C, fungal colonies with different morphological characters were picked up, and single spore isolation techniques were followed to obtain pure cultures of fungal isolates (Elwakil et al. 1990; Liyanage & Manage 2016a). The isolated pure cultures were maintained at 4°C on slants

containing PDA and subcultured once a fortnight.

**2.6 Identification of isolated fungi species**

Morphological features of the colony, including its colour on both sides of the culture plates, shape, elevation, and the nature of the margins, were observed. Fungal species that showed pathogenicity on water hyacinth were identified using the adhesive tape culture method (Hughes et al. 2004).

**2.7 Measuring the effect of isolated fungi on water hyacinth**

Pathological effects on single-leaf and whole-plant pathogenicity tests were used to determine the effect of isolated fungi on water hyacinth

**2.8 Pathological effect on a single leaf**

Fresh leaves of water hyacinth (whole plant) with more or less the same surface area were collected, and three scratch marks (1-2 mm long) were made on each leaf using a sterilised inoculation needle. A small mass of fungal mycelia and spores from the fungal cultures was applied to the scratched part of each leaf using an inoculation needle. The control leaves were maintained with scratch marks without any fungal application in a separate tank. Samples were incubated at 28 0C for 36 hrs (Ray & Hill 2012). Daily observation was made, and disease symptoms were recorded for 3 days. Pathogenic response was rated according to the width of the lesion as: ++ >5mm (Highly pathogenic), + > 5-3mm (Pathogenic), ->3mm (Nonpathogenic). The same method was followed for roots and petioles as well (Ray & Hill 2012).

**2.9 Whole plant pathogenicity tests (laboratory scale)**

Healthy water hyacinth plants were taken and sterilised using the method described above in isolation of fungi. Then matured fungal spores were sprayed on water hyacinth plants, while plants in the control tank received sterilised distilled water only. For a period of three weeks, the disease's severity was determined visibly, and the infection's ferocity was quantified using a score chart developed by Ray & Hill (2012).

**2.10 The combined effect of fungal pathogens and Water hyacinth mite (*Orthogalumna terebrans*) on water hyacinth**

The combined impact of individual pathogenic fungal species and the water hyacinth mite on water hyacinth plants was also assessed. During the study, it was identified that the mite effectively damaged the leaf. Thus, water hyacinth plants were exposed to 50 mites /plant and allowed for a week to get damage on leaves. During the study period, tanks were covered with nets in order to prevent mites from escaping the experimental tanks. After a week, cultured fungal spores were sprayed on water hyacinth plants. The control set up received sterilised water spray instead of fungal sprays and was kept in the same environmental conditions. Fungal pathogenicity and the number of mites per plant were noted daily for 4 weeks for each treatment setup.

## 3 RESULTS AND DISCUSSION

Various studies on isolation, identification, and pathogenicity of fungi associated with water hyacinth in its native range, as well as infested areas of the world, have been recorded (Okunowo et al. 2013; Ray & Hill 2012; Ray & Hill 2013). However, none of the studies reported on the isolation and identification of pathogenic fungal species infecting water hyacinth in Sri Lanka. Thus, information and application potential of fungal species as a biological control agent for water hyacinth within the native region of the country is timely and important, as the spread of water hyacinth in irrigation tanks is increasing.

### 3.1 Damage caused by water hyacinth mite

The water hyacinth mites (figure 1a) produce characteristic feeding galleries or tunnels extending towards the tip of the leaf and between the leaf veins (figure 1b). The galleries reach a length of 5-10mm. Small holes occur when mites come out of tunnels. Consequently, due to the loss of chlorophyll, leaves wilt, and finally necrosis occurs (Figure 1c). Damage done by water hyacinth mites was recorded as 11.6±0.7% of the total leaf area after four weeks of introduction of 50 mites per plant.

The present study is the first study in Sri Lanka that reports *Orthogaluma terebrantis* (water hyacinth mite) that can be found naturally within the country, and the intensity of the impact on water hyacinth under controlled conditions. Damage done by the water hyacinth mite on water hyacinth leaves was measured as 11.6±0.7% of the total leaf area after four weeks of the introduction of 50 mites per plant. The severity of the damage caused by the mite was detected as mild damage to the plants. This result demonstrates that the herbivory of *O. terebrantis* had little effect on water hyacinth growth, similar to the study of Maliu (2001). However, these results deviate from the field studies of water hyacinth mites done by Cordo & DeLoach (1976) reported that water hyacinth mites caused serious damage to water hyacinth plants in the field. The reason for this deviation can be the density of mites used for the study being less than that from the field condition and therefore it is possible that plants damaged by mites initially compensate by increasing their leaf turnover (Marlin et al. 2013).

**Figure 1.** Damage caused by water hyacinth mite on water hyacinth leaves (a: Water hyacinth mite, b: feeding galleries on leaf, c: necrosis effect)

### 3.2 Pathogenicity of isolated fungi

In the present study, 28 fungal species were isolated, and all were tested for their susceptibility to infect water hyacinth plants in vitro. Out of 28 fungal isolates, only 6 species were shown to have potential pathogenicity on water hyacinth (Table 1) and identified as *Trichoderma* sp., *Penicillium* sp., *Exserohilum* sp., *Alternaria* sp., *Fusarium* sp. and *Aspergillus* sp. (Figure 2). The selected potential fungal species were subjected to further experimental studies.

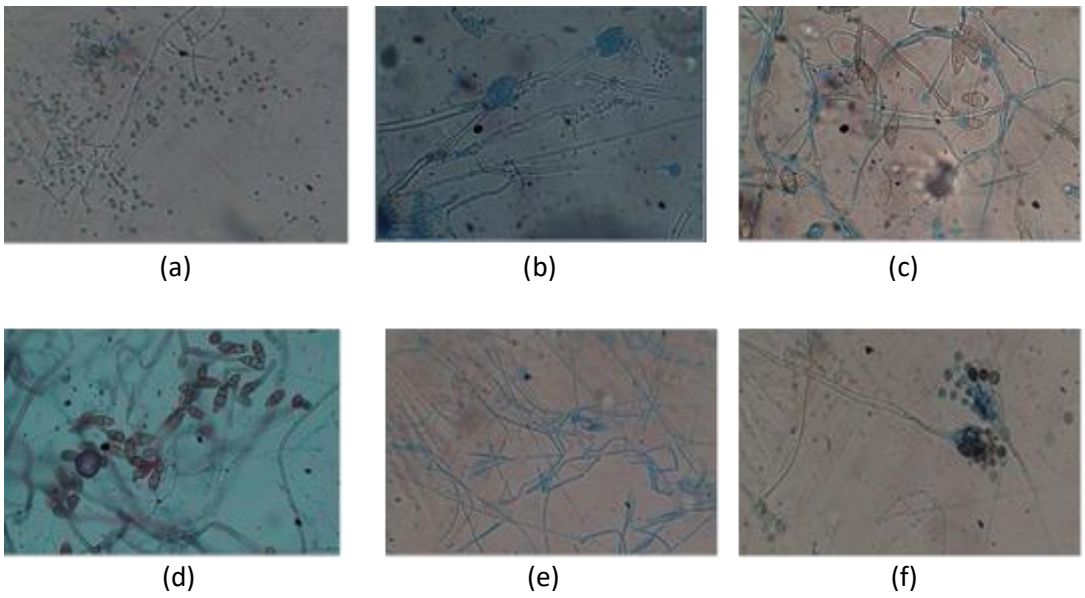


**Figure 2.** Identified potential pathogenic fungal species on water hyacinth (a: *Trichoderma* sp., b: *Penicillium* sp., c: *Exserohilum* sp., d: *Alternaria* sp., e: *Fusarium* sp., f: *Aspergillus* sp.)

**Table 1**: Pathogenicity of isolated fungal species

| Fungal species | Source of isolation | Pathogenicity |
|---|---|---|
| *Trichoderma* sp. (S-W) | root | + |
| *Penicillium* sp. (S/S/01) | leaves | + |
| *Exserohilum* sp. (S-S-04) | soil | ++ |
| *Alternaria* sp. (S-WP/06) | leaves | ++ |
| *Fusarium* sp. (S-WP/08) | soil | ++ |
| *Aspergillus* sp. (S-WP/10) | petioles | + |

*Key: ++ - Highly Pathogenic, + - Pathogenic, - - Non-pathogenic*

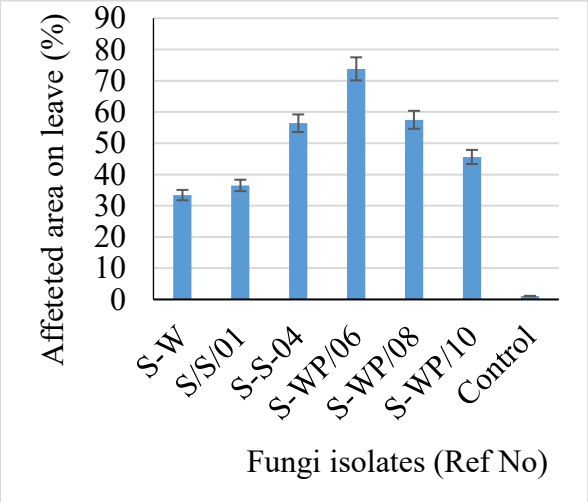


**Figure 3.** Pathogenicity percentage on leaves of water hyacinth after fungal inoculation

Figure 3 illustrates the percentage of affected surface area by each fungus on water hyacinth. Among the fungal isolates, *Alternaria* sp. (S-WP/06) showed maximum damage (73.8±7.3%) of leaf surface area. It was found that *Fusarium* sp. (S-WP/08) and *Exserohilum* sp. (S-S-04) had a considerable effect, which represented 57.5±4.8% and 56.4±2.7% respectively. Less than 50% effect was recorded by *Aspergillus* sp. (S-WP/10) (45.6±1.7%), *Penicillium* sp. (S/S/01) (36.5±3.7%), and *Trichoderma* sp. (S-W) (33.4 ±1.4%) isolates, respectively (Figure 3). The controls showed no damage to water hyacinth during the incubation. Species showing maximum damage were used for further studies.

Each of these three genera of fungal isolates was recorded as phytopathogenic over water hyacinth in previous studies (Conway et al. 1974; Evans & Reeder 2000). However, *Exserohilum* sp. was recorded only in the USA

and South Africa, and its pathogenicity on water hyacinth plants was not well documented (Conway et al. 1974; Evans & Reeder 2000). Disease symptoms shown by *Exserohilum* sp. included leaf spots and blight similar to those recorded by Conway et al. (1974). *Exserohilum* sp. was not previously recorded as a pathogenic fungal species on water hyacinth plants in Sri Lanka and in other tropical countries. Thus, the present study is the first study that records *Exserohilum* sp. as a potential biological control over water hyacinth plants in the Asian region.

The study found that the *Alternaria* sp. showed the highest pathogenicity (73.8±7.3%) among the three fungal strains, and the disease symptoms included leaf blight and lesions on both older and younger leaves, indicating the potential of using the strain as a biological control agent. Previous studies have also reported that fungal species belonging to the genus Alternaria are biological control agents for water hyacinth, and their pathogenicity has been recorded as 55-68% against water hyacinth (Shabana et al. 1995; Hill & Olckers 2000). This variation in pathogenicity can be due to the variation of climate conditions of countries where the studies were conducted (Hill & Olckers, 2000). Alternaria sp. has also been recorded to be associated with leaf spot disease of water hyacinth plants in Sri Lanka. However, studies of using Alternaria sp. as a potential biological control agent for water hyacinth were not well documented (Hettiarachchi et al. 1983). The present study has identified *Alternaria* sp. as a potential biological control agent for water hyacinth within Sri Lanka.

Fungal isolates of *Fusarium* sp. have also shown pathogenicity over 50% of the leaf surface area, indicating the potential of using them as biological control agents for water hyacinth. Disease symptoms included large leaf blight on older leaves, leaf spots on younger leaves and large irregular lesions on the petioles, which were observed after 28 days of fungal inoculation. Ray & Hill (2013) have studied the fungal strains associated with water hyacinth and have isolated fungal species belonging to the *Fusarium* genus, which have the potential to be used as biological control agents over water hyacinth. Reported disease symptoms ofthe isolated Fusarium sp. also included leaf blight and leaf spots. *Fusarium* sp. has also been recorded in Sri Lanka in association with the leaf spot disease of water hyacinth plants (Hettiarachchi et al. 1983). The present study has identified *Fusarium* sp. as a potential biological control agent for water hyacinth, as it has shown pathogenicity of 57.5±4.8% of the total leaf area.

### 3.3 Integrated effect of pathogenic fungi and water hyacinth mites on water hyacinth

**Table 2.** Severity of pathogenicity along with water hyacinth mite and fungal isolates

| Fungal species | Percentage of affected area (%) |
|---|---|
| *Trichoderma* sp. (S-W) + M | 44.7 ±2.3 |
| *Penicillium* sp. (S/S/01) + M | 48.9 ±3.4 |
| *Exserohilum* sp. (S-S-04) + M | 75.3 ±4.8 |

| | |
|---|---|
| *Alternaria sp. (S-WP/06) + M* | 92.8 ±7.6 |
| *Fusarium sp. (S-WP/08) + M* | 78.5 ± 5.2 |
| *Aspergillus sp. (S-WP/10) + M* | 56.8 ±6.1 |

M; Water hyacinth mite

Table 2 shows the disease severity of fungal species when applied to water hyacinth mites. More than 70% leaf damage was recorded by *Alternaria* sp. (S-WP/06), *Fusarium* sp. (S-WP/08) and *Exserohilum* sp. (S-S-04) along with water hyacinth mite. Among them, *Alternaria* sp. (S-WP/06) showed maximum damage (92.8 ±7.6%) followed by Fusarium sp. (78.5%), *Exserohilum* sp. (75.3%), *Aspergillus* sp. (56.8%), *Penicillium* sp. (48.9%) and *Trichoderma* sp. (44.7%) respectively at the end of the 4 weeks (Table 2).

Because water hyacinth mites may not affect the growth of the plants effectively on their own, the use of multiple agents has been found to increase the stress on the plants and reduce their growth more than when a single agent is used (Charudattan et al. 1978, Jimenez & Balandra 2007; Ray & Hill 2013; Ray & Hill 2016). The use of an integrated approach of two biological control agents for controlling the weed was not recorded in Sri Lanka, although other countries have used this approach for several decades (Coetzee et al. 2011). So far, only the two weevils, *N. eichhorniae* and *N. bruchi,* were studied as biological control agents in Sri Lanka (Julien 2000; UNDP 2017).

According to the results of the present study, the disease severity of each isolated fungal strain was increased when it was integrated with water hyacinth mite. This can be due to the occurrence of feeding galleries, which emerge as holes made by the adults, and pinholes created by the female mites to lay their eggs, allowing the phytopathogenic fungi to penetrate the internal tissues of the plants (Ray & Hill 2013).

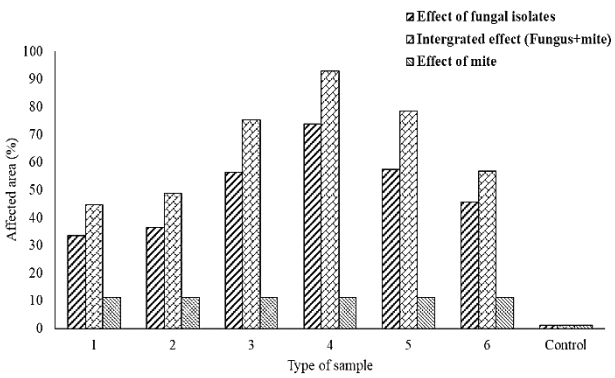


**Figure 4.** Comparison of the severity of pathogenicity of the mite, the pathogenicity of fungal isolates, and the integrated effect of water hyacinth mite with isolated fungi species [Fungal species:- 1: S-W, 2:S/S/01, 3:S-S/04, 4:S-WP/06, 5:S-WP/08, 6-S-WP/10]

Variation in severity of pathogenicity on water hyacinth plants by fungal species and mite along an integrated effect was evaluated (Figure 4). The least damage was found when applying water hyacinth mite alone (11.6±0.7%) after 28 days. Damages caused by fungal species varied from 73.8±7.3% to 33.4 ±1.4%. Integrated effects of each fungal species along with water hyacinth mite showed the highest to lowest disease severity from 92.8 ±7.6% to 44.7 ±2.3, respectively, with *Exserohilum* sp., *Alternaria* sp., and *Fusarium* sp.

Damage caused by *Exserohilum* sp. was less than 75% of the leaf surface area. This can be due to its poor disseminating capacity, as

reported by Zhang & Watson (1997) and was also apparent in field studies where plants infected by *Exserohilum* sp. were rare in natural conditions. However, in the present study, all isolated fungal strains have increased their pathogenicity after integration with water hyacinth mites. In accordance with previous studies, *Exserohilum* sp., *Alternaria* sp. and *Fusarium* sp. associated with water hyacinth and water hyacinth mites have shown their potential of being used as biological control agents of water hyacinth in Sri Lanka.

Thus, it has provided evidence for an effective approach to the management of water hyacinth in a sustainable manner as proven by previous studies around the world (Coetzee et al. 2011; Cordo 1998).

## 4. CONCLUSION

The findings of this study underscore the effectiveness of an integrated approach involving water hyacinth mites and phytopathogenic fungi as a viable strategy for controlling the invasive water hyacinth. This combined approach exhibits greater potential compared to the use of individual pathogenic fungal agents. The results highlight the importance of considering diverse biological control methods for tackling the challenge posed by water hyacinth invasion. Further research and field trials are recommended to validate the feasibility and long-term efficacy of this integrated approach on a larger scale.

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