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EFFICACY OF HERBICIDES AND SHADING METHODS TO CONTROL INVASIVE *SPHAGNETICOLA TRILOBATA* (L.) PRUSKI

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Abstract

Sphagneticola trilobata (L.) Pruski is one of the most noxious invasive plant species, posing a serious threat to native biodiversity. This species has been invading large areas of Bangladesh for the last two decades. The study investigated the efficacy of herbicides and shading treatments during winter and monsoon to find an effective control method for *S. trilobata*. Both surface-inhabiting (Glyphosate) and root-inhabiting (Paraquat) herbicides were applied for six months in five different combinations (100% Glyphosate, 75% Glyphosate + 25% Paraquat, 50% Glyphosate + 50% Paraquat, 25% Glyphosate + 75% Paraquat, and 100% Paraquat) and the percentage coverage was measured for each experimental plot. Results from the study showed that all shading and chemical combinations of Glyphosate and Paraquat significantly reduced the percentage coverage of *S. trilobata* in each experimental plot ($p < 0.05$). However, the shading and Paraquat-dominated combinations reduced the coverage slowly compared to the Glyphosate-dominated ones. Among five different combinations, Paraquat-dominated herbicides were less effective in controlling the species. On the other hand, the Glyphosate-dominated combinations completely eradicated the species within a week but increased thereafter. Meanwhile, the seasonal variation was found to be highly significant ($p < 0.0001$) among treatments, and a significant reduction was observed in winter compared to the monsoon. The results of this study highlighted that the Glyphosate-dominated combinations were the fastest, while the Paraquat-dominated combinations and shading were slower control methods.

Keywords: Invasive plant, *Sphagneticola trilobata*, shading, herbicides, seasonal variations

Introduction

Sphagneticola trilobata (L.) Pruski [syn. *Wedelia trilobata* (L.) Hitchc.] is considered one of the most notorious invasive plant species on Earth. IUCN has listed it among the 100 world's worst invasive species (Lowe et al., 2000), and the Florida Exotic Plant Pest Council (FLEPPC) classified it as a category II invader (FLEPPC, 2020). It is a creeping and mat-forming perennial herb native to Central America and has already invaded the tropics and subtropics as an ornamental plant (Qin et al., 2015; Hossain & Hassan, 2005). It spreads vegetatively, forms a dense ground cover, and once established, it crowds out other species (Wagner et al., 1990; Csurhes & Edwards, 1998; Wagner & Van Driesche, 2010). It also becomes naturalized along streams, canals, borders of mangroves, and coastal strands (Thaman, 1999; Csurhes & Edwards, 1998). Apart from its invasive nature, some strong allelopathic evidence has also been found in agricultural crops like potatoes, tomatoes, and rice (Nie et al., 2004). Qin et al. (2015) reported that it prefers hot and humid environments and can occur in areas with different environmental conditions than those experienced in its native range. Due to aggressive growth habits, tolerance to environmental stresses, and the capacity to synthesize allelochemicals, *S. trilobata* can invade rapidly (Wang et al., 2012). Moreover, it can significantly reduce the germination capacity of competing plants up to ~72% especially dicots (Hernández-Aro et al., 2016).

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Through aggressive nature and allelopathy, it reduces significant amounts of food crops to inhabit biological diversity, especially for birds and insects (Linnell et al., 2009; Junhirun et al., 2012).

Globally several studies have been undertaken to control or eradicate this noxious invasive species through physical/shading treatments as an effective approach (Englberger, 2009; Junhirun et al. 2012; Qi et al. 2014; Pu et al. 2022). However, Pu et al., (2022) claimed that only uprooting is not enough to eradicate it fully as it can sprout from a single node. Chemical methods have also been tried for this species. Motooka et al. (2002) found a minimum of 2 lb/acre herbicide of dicamba, and 2,4-D was effective to kill *S. trilobata*. The plant is also sensitive to triclopyr, or Glyphosate (foliar spray) applied by the drizzle method but probably at 2 lb/acre or more (Englberger, 2009). Rolfe et al. (2019) suggested metsulfuron methyl herbicide (600g/kg) along with 10g/100L surfactant as an effective herbicide for this species in Australia. Wu and Hu (2006) suggested 1:600 picloram and clopyralid in 10 days and 1:800 fluroxypyr in 15 days as the most effective herbicides to eliminate *S. trilobata* in China. In Bangladesh, it was first reported in Gulshan, Dhaka (Hossain & Hassan 2005), where it was initially planted for ornamental purposes. In an earlier study, Rahman (2013) found this species in northern Bangladesh. The introduction of this exotic species highlights the ineffective plant quarantine system (Rahman et al., 2014). However, there is a lack of information on the spread and distribution of this invasive species in Bangladesh. So far, it is already spread to natural habitats in the Northern (Rahman, 2013), central (Mia et. al., 2020), and southwestern regions (Rahman, 2015). It has also invaded municipality areas of Dhaka and Chittagong (Ahmed et al., 2007-09). So, Bangladesh is also highly susceptible to the threat of this invasive species and poses a high risk in consideration of long-term productivity and ecological sustenance (Kanatas et al., 2020; Rasmussen, 2004).

To control this alien exotic plant, chemical application is one of the most popular and frequently used methods globally (Green, 2014), despite a few drawbacks (Weidlich et al., 2020; Randy, 1998). However, the use of extensively used chemicals can pose less harm to the ecological balance, as indicated by several studies (Chen et al., 2017; El-Metwally & El-Wakeel, 2019). In many instances, studies have shown that commonly used chemicals may have lesser effects compared to newly introduced ones, thereby avoiding the challenge of introducing new chemicals to the environment (Dayan & Duke 2006, 2014). Therefore, it is advisable to utilize commonly used herbicides for plant control where necessary, and their combinations can be tested with other environmental factors such as temperature and precipitation (Alatürk et al., 2018). Globally, Glyphosate, also known as N-(phosphonomethyl) glycine, has been extensively used as an herbicide for root inhabitation for the past 40 years due to its minimal side effects (Duke & Powles, 2008; Van Bruggen et al., 2018). On the other hand, Paraquat, a bipyridinium herbicide, has been tested as an effective contact herbicide for surface application (Bromilow, 2004). Therefore, Glyphosate (41%) and Paraquat were applied to ensure maximum effectiveness (Vicente et al., 2001) while minimizing their impact on the soil (Hawkes, 2014). In Bangladesh, Glyphosate 41% and Paraquat are also the most used chemicals for herb control (Mustari et al., 2014).

For this instance, a control strategy is much needed to develop to reduce the destructive effects of *S. trilobata* on local vegetation. But the number of studies on controlling *S. trilobata* is very minimal and most of the studies suggested removing the topsoil for full elimination (Wu et al., 2013). To eradicate this problem, posed by this invasive species, the study aims to test two herbicides (Glyphosate and Paraquat) and shading methods to reduce the coverage of *S. trilobata* in both winter and monsoon seasons.

Materials and Method

Study area

The study was conducted at Khulna University Campus, located between 22º47′57″ N to 22º48′18″ N and 89º31′38″ E to 89º32′21″ E in the southwestern part of Bangladesh (Figure 1). Several areas of this university have already been invaded by *S. trilobata*, and the experimental plots were established in those patches. This area experiences a subtropical climatic conditions with a dry winter from October to March, humid summer from March to June, and a warm monsoon from June to October. However, the maximum and minimum temperatures vary throughout the year. In December-January, the temperature falls to its lowest point at 12-15ºC, while it reaches its highest in April-June at 41-45 ºC. July is the month with the maximum amount of precipitation, with 20-25 days of precipitation (BBS, 2014).

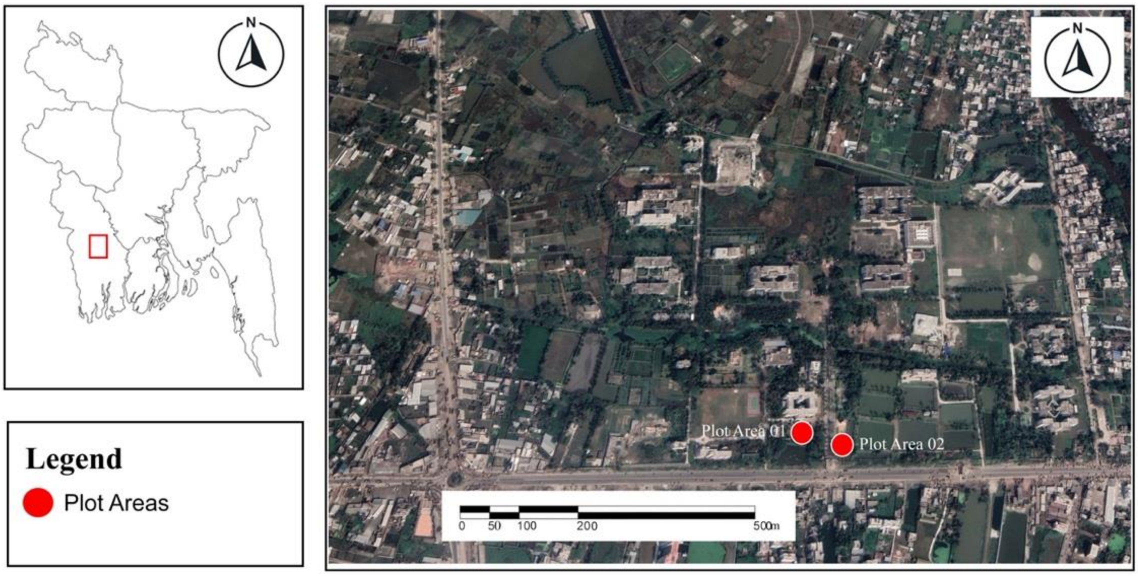

Figure 1. Study area in Khulna University, Khulna. The red dot indicates the experimental plot.

Experimental design

To apply the shading and herbicides treatments, 36 square experimental plots with dimensions of 1m × 1m were established at Khulna University Campus in Khulna, Bangladesh (Figure 1). As the vegetation of the area exhibited two distinct growing seasons, namely monsoon and winter, throughout the year, the treatments were replicated in both seasons to analyze the changes in coverage of the studied species. For the analysis of the herbicides and shading treatments, three replications were conducted in each season for all treatments. Therefore, a total of 18 sample plots (3 × 6) were studied, consisting of six different treatments (shading and five combinations of herbicides) for each season. For the shade treatment, the entire plot area was covered with an opaque PVC Vinyl sheet, and data were collected on a weekly basis for six weeks. However, for the chemical treatment, the percentage coverage was measured daily for three weeks. The observations were conducted based on the decreasing rate of coverage of the studied species. To measure the effectiveness of the herbicides, this study used the most readily available herbicides for surface application (Glyphosate 41%) and root application (Paraquat) (Figure 2). For applying the herbicides treatment, a 2% water solution of each herbicide (Glyphosate and Paraquat) were prepared and then mixed with different combinations. The applied mixing ratio of the herbicides are following: 1:0 (100% Glyphosate), 1:3 (75% Glyphosate + 25% Paraquat), 1:1 (50% Glyphosate + 50% Paraquat), 1:3 (25% Glyphosate + 75% Paraquat) and 0:1 (100% Paraquat). These treatments were replicated three times for both the monsoon season (May-June) and the winter season (December-January) to analyze the gradual decrease in cover (%) of the studied species.

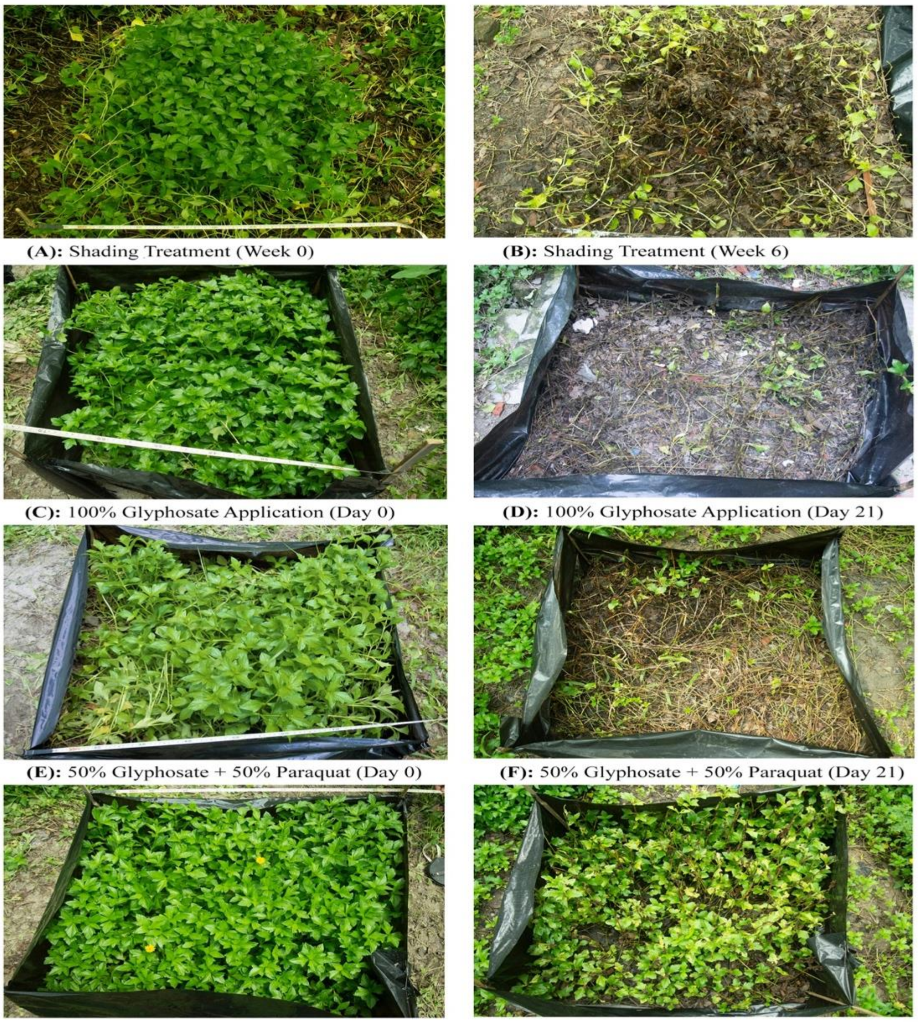

Figure 2. *S. trilobata* coverage in shading and herbicides treatment combinations.

Data Collection

The effectiveness of the shading and herbicides treatments was determined by analyzing the percentage of vacant areas, which were captured as images at specific time intervals (weekly for shading and daily for herbicides treatments). The plot areas were captured with a Nikon-D3200 camera with Nikkor 55–300 mm AF-S DX lens at a height of 1m, and calibration was maintained by placing a meter tape next to each sample plot. After capturing the images from each plot, the coverage was calculated using Image J (Version 1.6.0). To calculate the gradual decrease in coverage, the pixel error was set to less than 5%. Pixels exceeding this threshold were corrected for illumination gradients and converted to black and white (B&W) (16 bytes) using interactive threshold to mask the actual coverage (Figure 3).

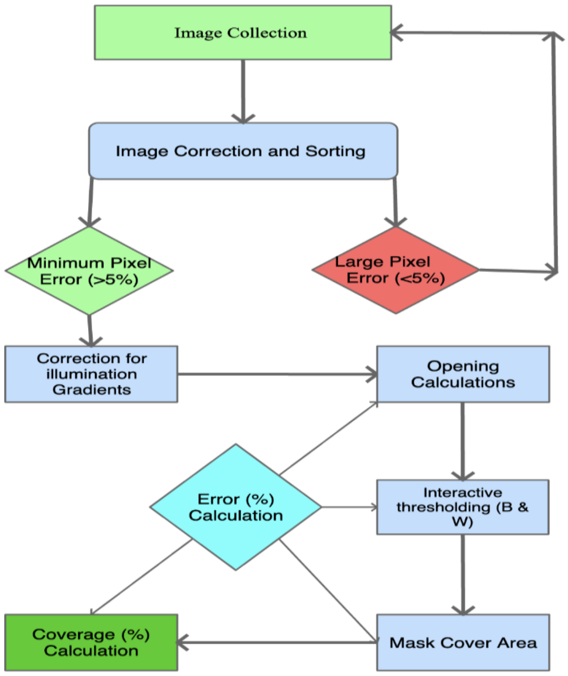

Figure 3. Flowchart showing steps involved with image analysis tool application (Image J) for the measurement of area coverage (%).

Statistical analysis

All statistical analysis and graphics were performed using R statistical software (version 4.0.4) (R Core Team, 2020). The normality of residuals and heteroscedasticity were assessed using the Shapiro-Wilk normality test from the 'R stats' base package and the studentized Breusch-Pagan (BP) test from the 'MuMIn' package (Fox & Weisberg, 2019; Bartoń, 2020). The percentage coverage data were subjected to an arcsine transformation before conducting the statistical tests, and subsequently back-transformed for graphical presentation. The cumulative coverage of each day/week and season, corresponding to a particular treatment, were analyzed using two-way/three-way analysis of variance (ANOVA) tests using with the 'car' package (Fox & Weisberg, 2019).

Results

Effect of shading on S. trilobata coverage

The shading treatment successfully decreased the coverage of *S. trilobata*. After five weeks, the percentage coverage decreased to 93% in the winter season, while in the monsoon, it was 88% (Figure 4). However, there was a slight increase again in week six. The two-way Analysis of Variance (ANOVA) test showed that both season and week had a significant impact on reducing the coverage of *S. trilobata* (Week, $F_{6, 28} = 554.41$, p <0.0001 and season, $F_{1, 28} = 47$ 31.55, p <0.0001) (Table A.1). However, there was no significant interaction between them.

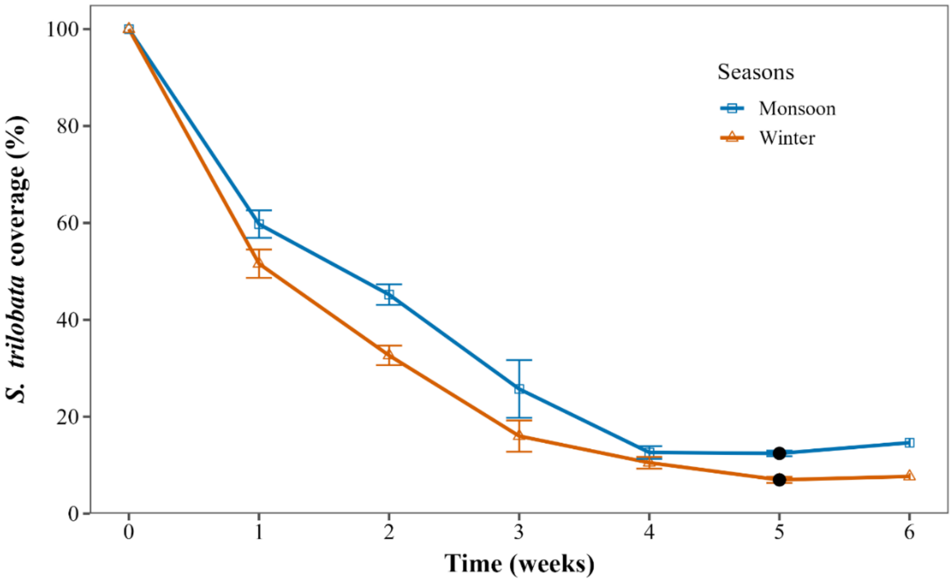

Figure 4. The percentage coverage of *S. trilobata* under shading treatment in monsoon and winter. The black dot indicates the lowest percentage coverage of *S. trilobata*.

Effect of herbicides treatment on S. trilobata Coverage

Paraquat (100%) showed minimal effect compared to the other four herbicide treatments. It only defoliated 10-13% in monsoon, while in winter, it reduced coverage up to 30-34% within 3 weeks (Figure 5E). In the case of a 1:3 solution (25% Glyphosate + 75% Paraquat), the minimum coverage (72%) was observed in the second week in the monsoon, while in winter, it took 3 weeks to reach the minimum (53%) (Figure 5D). An equal combination of glyphosate and paraquat (50% Glyphosate + 50% Paraquat) proved to be one of the most effective chemical combinations for retaining the lowest coverage. This 1:1 solution gradually reduced the leaf coverage to a minimum value of 21% in the monsoon, whereas in winter, there was a rapid decline in the coverage area, reaching 16% in the first week (Figure 5C). Among the different combinations of Glyphosate and Paraquat, a higher concentration of Glyphosate (25% Glyphosate + 75% Paraquat) accelerated the rate of declining cover within 1-2 weeks, but the yield was retained for a shorter period. For this specific treatment, the minimum coverage was recorded at 9% in the monsoon after two weeks of application. However, in winter, the decline rate was faster than in the monsoon, reaching the minimum value of 6% in the first week (Figure 5B). Among all treatments, pure Glyphosate (100%) application showed quick but less stable defoliation compared to other treatments. Pure surface application in the monsoon resulted in the maximum decrease in area coverage (2.7%) in the second week, while the minimum value in winter was recorded at 3.3% in the first week (Figure 5A).

The three-way ANOVA showed that the percentage of coverage of *S. trilobata* significantly decreased due to the application of the treatments up to twenty-one days in both seasons (days: $F_{21, 440} = 430.58$, p <0.0001, 172 seasons: $F_{1, 440} = 233.78$, p <0.0001 and herbicides treatment, $F_{4, 440} = 4318.54$, p <0.0001) (Table A.2 & A.3). The pairwise combination of days, seasons, and treatments also resulted in a significant reduction in the coverage of the studied species ($p < 0.05$). Additionally, the interaction effect of days, seasons, and treatments was found to be significant ($p < 0.05$).

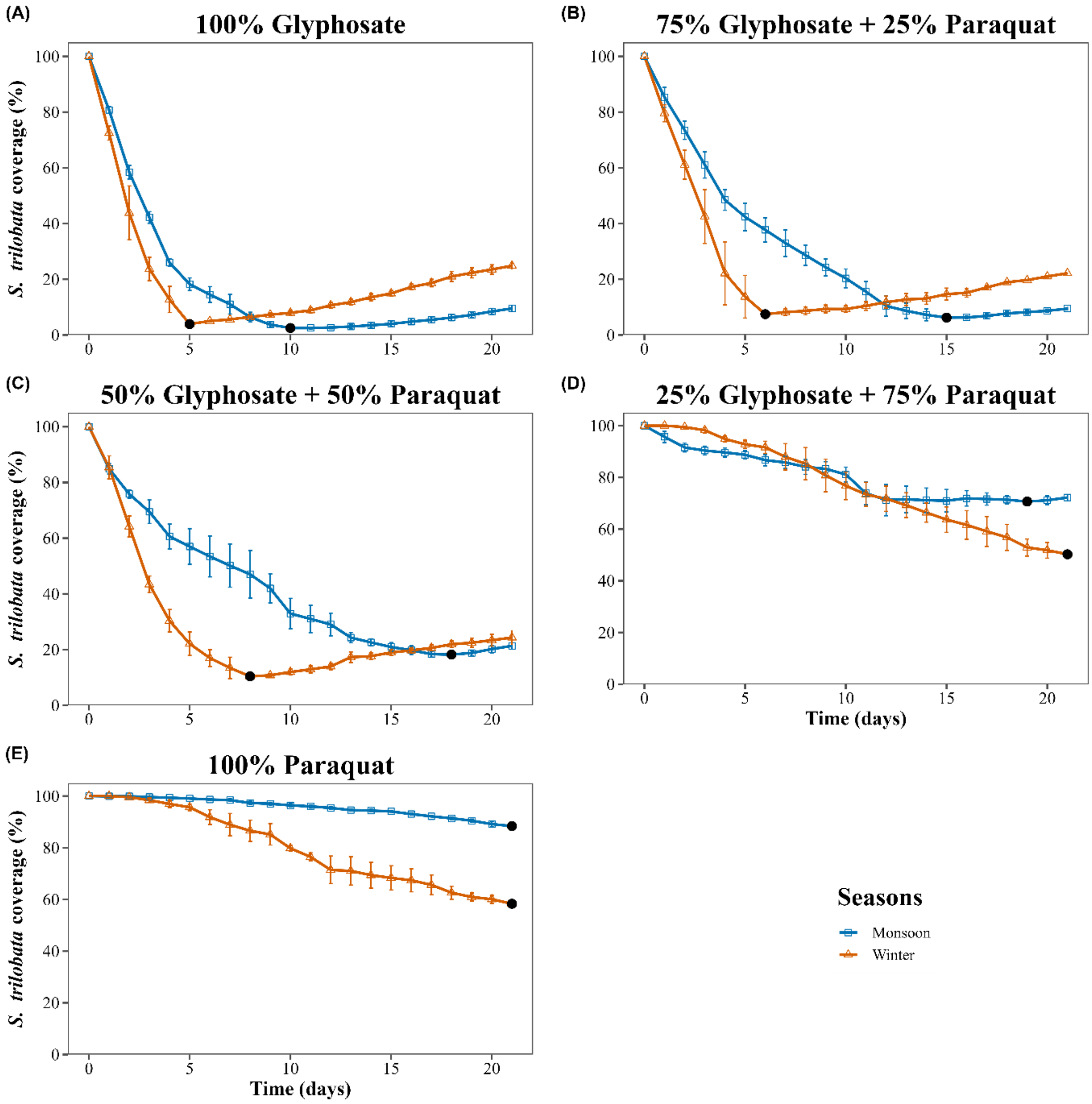

Figure 5. *S. trilobata* coverage in different combinations of herbicides treatment. The black dot indicates the lowest percentage coverage of *S. trilobata*.

Comparison among shading and herbicides treatment

The coverage of *S. trilobata* was monitored against shading treatments for six weeks and herbicides treatments for three weeks (Figure 6). The percentage coverage decreased by approximately 95% with Glyphosate dominated combination within one week in winter, but it started to increase again afterward. On the other hand, both shading and Paraquat dominated combination showed a slower decreasing rate, and neither showed any increasing trend after three weeks. The three-way ANOVA showed that the percentage of coverage of *S. trilobata* significantly decreased from the beginning of the treatments until three weeks in both seasons and across all six treatments (weeks: $F_{3, 96} = 2341.99$, $p < 0.0001$, seasons: $F_{1, 96} = 55.0$, $p < 0.0001$ and treatments, $F_{5, 96} = 533.47$, $p < 0.0001$) (Table A.3). The pairwise combination of weeks, seasons, and treatments also resulted in a significant reduction in the coverage of the studied species ($p < 0.05$). Additionally, the interaction effect of weeks, seasons, and treatments was found to be significant ($p < 0.05$).

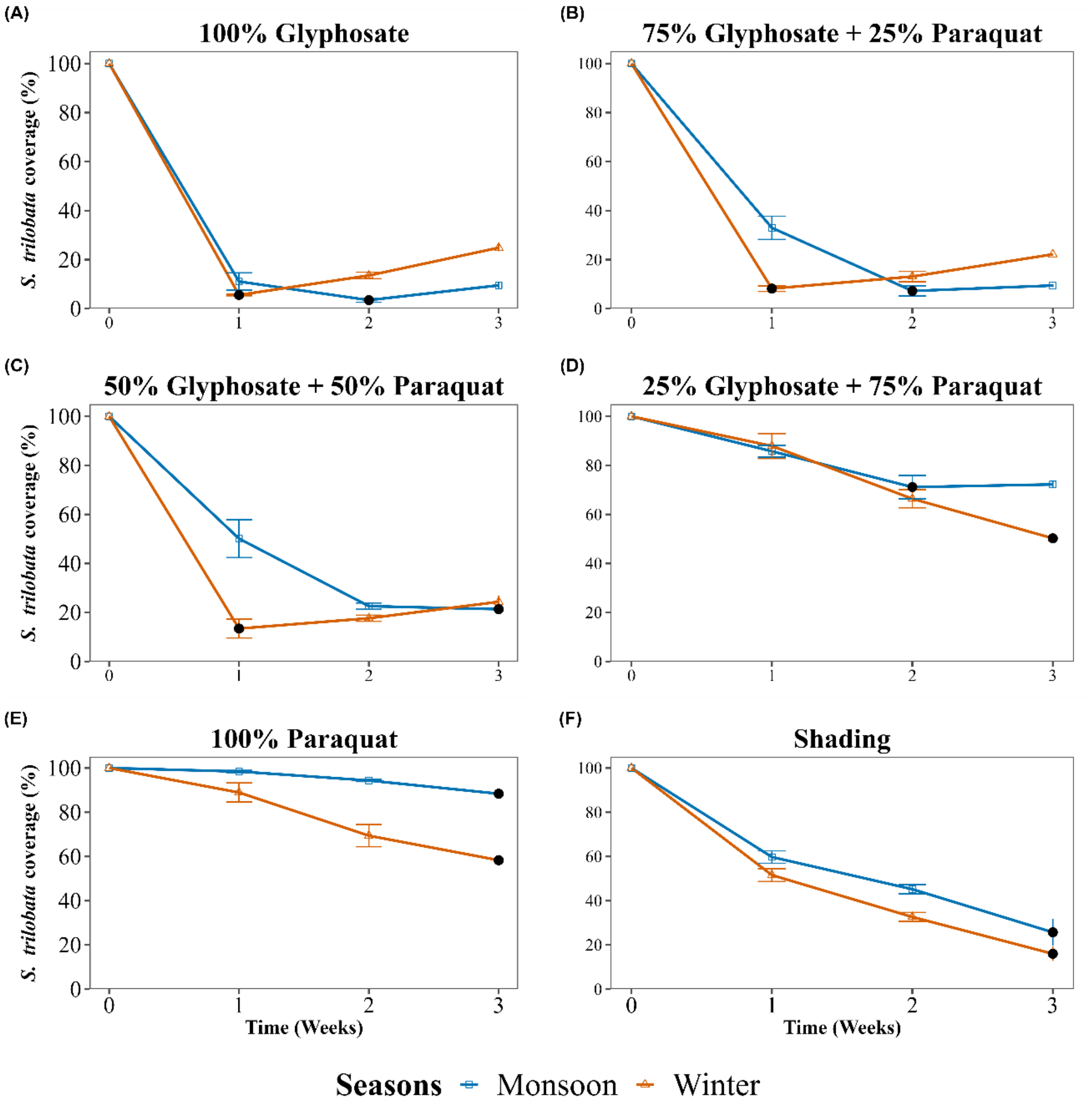

Figure 6. *S. trilobata* coverage in shading and herbicides treatment combinations. The black dot indicates the lowest percentage coverage of *S. trilobata*.

Discussion

The present study investigated the effect of shading and chemical herbicides in controlling the noxious invasive species, *S. trilobata*, in Bangladesh. Irrespective of the combinations, all six treatments successfully eradicated the coverage of the studied species. However, the shading treatments showed better results compared to Paraquat herbicides. Due to their environmental friendliness, shading methods can be a viable alternative to chemical approaches. Shading exhibited consistent and favorable reductions over time, achieving a decline of up to 21% in coverage during winter within three weeks. In addition to the growing popularity of biological control methods for invasive species (Müller-Schärer et al., 2004; De Clercq et. al., 2011), several other chemicals and shading control methods have been recommended for selected species in a controlled environment (Weidlich et al., 2020; Schooler et al., 2010). Consistent with the findings of Wu et al. (2013), our study also observed that the coverage of *S. trilobata* can be reduced up to a maximum of 97% but achieving complete (100%) elimination is challenging. The retention of that minimal leaf coverage lasts only 12-19 days (Figure 5 & 6), after which *S. trilobata* begins to regrow.

Herbicides have a significant impact on plant physiological processes, particularly in terms of restricting nutrient and water uptake from the soil, which can lead to tissue damage (Belgers et al., 2007) and the destruction of root microbe associations (Thetford & Gilliam, 1991). However, plants possess a high degree of flexibility and adaptability to cope with root stress, and alternative methods can be employed to minimize its effects (Pan, 2001). In this study, it has been observed a similar pattern of slow response for root applications (Figure 5A-5C). For example, root applications or mixtures dominated by Paraquat (100% Paraquat, 25% Glyphosate + 75% Paraquat, 50% Glyphosate + 50% Paraquat) required a longer time to decrease leaf coverage compared to Glyphosate-dominated mixtures. On the other hand, chemicals that primarily interact with the plant surface or tissues, such as Glyphosate, have a quick impact (usually within 15 days) on plant tissues (Grillo et al., 2014). Similarly, we observed a rapid response from Glyphosate-dominated applications, whereas the Glyphosate (100%) mixture required less than a week for significant effects to be observed (Figure 6F).

All treatments exhibited a significant decrease in coverage area during winter compared to the monsoon season. Overall, winter accelerated the reduction of leaf coverage for both shading and herbicides treatments. Therefore, applying control measures for this species during winter would be preferable in tropical and subtropical environments. The seasonal effects, particularly the environmental gradients, had a notable influence on the rapid decline of leaf coverage observed in this study, which aligns with findings from a previous study (Morais et al., 2021).

Among six treatments, the shading treatment and Paraquat-dominated combinations exhibited a slow reduction in the coverage area. On the other hand, the Glyphosate-dominated combinations showed the fastest decrease in leaf coverage within one week. After applying the herbicides treatments, the Glyphosate-dominated combination (100% Glyphosate and 25% Paraquat + 75% Glyphosate) yielded better results compared to the Paraquat-dominated combinations (100% Paraquat and 25% Glyphosate + 75% Paraquat), which is consistent with earlier studies (Mat et al., 2006; Patras & Artenie, 2007; Ada et al., 2012). In the case of 100% Glyphosate application, the leaf coverage decreased at the lowest value of 2.7% both in winter and monsoon. This herbicide provided rapid results due to its ability to swiftly reduce coverage. Conversely, a higher concentration of Paraquat application showed minimal effects (62% in the monsoon) on coverage and required the longest duration (over 21 days in winter) to take effect, making it less effective than the shading treatment.

Finally, for a control method to be considered viable, it is crucial to suppress plant regrowth for at least one season of the year (Keane & Crawley 2002; Mandle et al., 2011). However, the treatments applied in this study did not demonstrate sustained effects beyond 3 weeks for chemical treatments and 6 weeks for shading treatments. As a result, this study cannot recommend any treatment for long-term control of the studied species. Future studies should investigate the control measures over longer time intervals. Additionally, it is recommended to explore other potential shading methods, such as topsoil removal and controlled fire, to enhance the efficacy of shading control methods.

Conclusion

To eradicate the invasive creeping plant, *S. trilobata*, this study employed both shading and herbicide treatments to decrease the percentage of coverage in both monsoon and winter seasons. The results demonstrated that both shading and all five combinations of chemical herbicides effectively reduced the area covered by the studied species. Among the six treatments, the shading treatment and Paraquat-dominated mixtures exhibited a slower reduction in coverage. However, the shading treatment yielded faster results compared to the Paraquat-dominated combination. Additionally, all treatments showed a significant reduction in coverage during the winter season compared to the monsoon season. These findings highlighted that Glyphosate-dominated combinations offer the fastest method of reducing coverage while shading and Paraquat-dominated mixtures are comparatively slower in their effects.

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Conflict of Interest
The authors declare no conflict of interest

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Annex

Table A.1: Two-way ANOVA results for percentage coverage with shading treatments in different weeks and seasons.

Source	DF	SS	MSS	F	p
Weeks	6	7.3	2.1	554.41	<0.0001
Seasons	1	0.70	7.9	31.55	<0.0001
Weeks * Seasons	6	0.02	0.2	1.56	> 0.05
Residuals	28	35.5	0.06		

Table A.2: Three-way ANOVA results for percentage coverage with chemical treatments in different days and seasons.

Source	DF	SS	MSS	F	p
Days	21	38.82	1.85	430.58	<0.0001
Seasons	1	1.00	1.00	233.78	<0.0001
Herbicides Treatments	4	74.16	18.54	4318.54	<0.0001
Days * Seasons	21	0.83	0.04	9.19	<0.0001
Days * Herbicides Treatments	84	6.39	0.08	17.72	<0.0001
Seasons * Treatments	4	1.73	0.43	100.47	<0.0001
Days * Seasons * Herbicides Treatments	84	3.32	0.04	9.21	<0.0001
Residuals	440	1.89	0.004		

Table A.3: Three-way ANOVA results for percentage coverage with shading and herbicides in three weeks and seasons

Source	DF	SS	MSS	F	p
Weeks	3	21.79	7.26	2341.99	<0.0001
Seasons	1	0.17	0.17	55.0	<0.0001
Herbicides Treatments	5	8.27	1.65	533.47	<0.0001
Weeks * Seasons	3	0.16	0.05	17.30	<0.0001
Weeks * Herbicides Treatments	15	3.37	0.22	72.37	<0.0001
Seasons * Herbicides Treatments	5	0.31	0.06	19.89	<0.0001
Weeks * Seasons * Herbicides Treatments	15	0.56	0.04	11.88	<0.0001
Residuals	96	0.30	0.003		