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Allelopathic effect of different solvent extracts of *Turnera subulata* Sm. leaves against selected plants and weed

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Abstract

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Allelopathy had been identified as an alternative in minimize the dependency of herbicide in controlling weed. There has been limited research performed on *Turnera subulata* Sm., despite the exploration of allelopathy in the Passifloraceae family. In this research, the allelopathic effect of *T. subulata* leaf extracts was investigated on bioassay species of mustard, wheat grass, and weedy rice under laboratory conditions using various solvents, including water, methanol, ethyl acetate, and hexane. The experiments were conducted in different concentrations; 0.1, 1, 10, 50, and 100 mg/ml leaf extracts in five replicates. The total phenolic and flavonoid compounds of both leaf extracts were also determined. *T. subulata* leaf extracts significantly reduced the growth of bioassay species at 50 and 100 mg/ml. At 100 mg/ml, off-white *Turnera* aqueous extract suppressed the most in root length of mustard, followed by the yellow *Turnera*, with 92% and 89% of inhibition, respectively, compared to the control. Aqueous extracts had the strongest inhibitory effect on the root growth of all species. The degree of phytotoxic activity of different solvent extracts on both yellow and off-white *T. subulata* can be classified in the order of decreasing inhibition as follows: water > methanol > ethyl acetate > hexane. When evaluated at 0.1 and 1.0 mg/ml of all solvents, there was a substantial stimulatory effect on the root and shoot growth of the bioassay species. The allelopathic activities of *T. subulata* leaf extract greatly depended on extract concentrations, bioassay species, and solvent types. These findings can be used as a benchmark for future weed management strategies.

Keywords: Turnera subulata Sm.; Allelopathy; Solvent Extract; Total Flavonoid Content; Total Phenolic Content

Introduction

Farmers face challenges in controlling weeds, which are non-commercial plants that interfere with crop growth and reduce crop production by consuming light, moisture, and space (Ani et al., 2018; Guglielmini et al., 2017; Mushtaq & Siddiqui, 2018). Chemical methods were once the most effective way to control weeds in crop fields (Duke & Dayan, 2018), but their use is now problematic due to environmental and health risks (Bajwa et al., 2020). Furthermore, the overuse of synthetic herbicides has resulted in the emergence of 262 herbicide-resistant weeds, including 152 dicot and 110 monocot species (Anh et al., 2021; Maclaren et al., 2020).

Therefore, there is a need for sustainable weed management, which can reduce the reliance on chemical herbicides (Scavo et al., 2020). Effective and safer weed control can be

achieved by cultivating allelopathic plants or placing allelopathic material adjacent to weeds, leading to the accumulation of allelochemicals (Michael et al., 2016). Allelochemicals show potential as a new type of herbicide, as they not only help manage weeds but also protect plants from harm caused by microbes, herbivores, and competition with other plants.

Allelopathy is the term used to describe any direct or indirect effects that one plant, including microorganisms, has on another by releasing chemical compounds into the environment to affect the growth and development of nearby plants (Bahadur et al., 2015; de Albuquerque et al., 2011; Rice, 1984). Numerous allelochemicals, including glucosinolates, phenolic acid, fatty acids, isoflavonoids, scopoletin, hydroxamic acids, benzoxazinoids, dhurrin, and sorgoleone were used to control weed (Jabran, 2017). Therefore, the use of allelochemical is a promising approach for sustainable weed control in agriculture (Dahiya et al., 2017). This may result in the discovery of new, natural herbicides in the future (Kong et al., 2019).

Sulistiyani et al. (2022) emphasize the importance of accurately identifying and classifying species in scientific studies. Traditional taxonomy, which is based on physical characteristics, can be difficult to distinguish between closely related species, especially in complex cases. Thus, based on molecular identification using the ITS2 primer, both species of *T. subulata* (off-white and yellow) were identified. *Turnera subulata* Sm. from the Passifloraceae family was successfully identified as the off-white and yellow *Turnera*. The off-white *Turnera* has white petals, while the yellow *Turnera* has yellow petals, both with a dark base (NParks Flora & Fauna Web, 2022). The organoleptic properties and different colors of plants are due to phytochemical compounds (Saravanan et al., 2018).

T. subulata is an herbaceous subshrub species primarily found in the subtropical and tropical regions (Solis Neffa & Fernandez, 2000) Indonesia, Malaysia, Florida as well as several Pacific Islands (Saravanan et al., 2018). This plant is a perennial herb with a thick taproot and a woody, cylindrical stem with simple leaves (de Brito Filho et al., 2014). *T. subulata* was used in folk medicine due to its pharmacological properties, such as anti-inflammatory, hypoglycemic, antifungal, and antioxidant activity (Kumar et al., 2005; Rocha et al., 2018).

The current research has been examining the use of medicinal plants because these plants have more biologically active substances in their secondary metabolites, which makes them capable of inhibiting the growth and development of other plants by acting as allelochemicals (Islam et al., 2018). The active ingredients of potentially allelopathic plant species benefit from medicinal properties activities (Mirmostafaee et al., 2020).

According to a study by Luz et al. (2022), the phytochemical analysis of *T. subulata* revealed the presence of various bioactive compounds such as flavanols, alkaloids, tannins, cyanogenic glycosides, fatty acids, triterpenoids, and phenolic compounds. The study discovered three phenolic compounds in the flower and leaf extract of *T. subulata*, including vitexin-2-O-ramnhoside, 7-O-beta-glucopyranosyl-4tract of s, alkaloids, tannin, and ferulate. The study by Badrulhadza et al. (2018) recorded that *Turnera* species can attract almost 28 insects, which suggests that there may be certain allelochemicals present in *T. subulata* that are attracting these insects. However, the specific allelochemicals responsible for attracting insects have not been identified in this study.

In this study, Follin-Ciocalteau assay and aluminium chloride colorimetric assay was examined to determine the phenolic and flavonoid compound respectively. The leaf extract of off-white and yellow *Turnera* were extracted to screen the total phenolic compound and total flavonoid compound. Hussain et al. (2020) stated that the presence of phenolic and flavonoid compounds found in plants are known to play a role in allelopathic interactions. Studies have shown that higher levels of phenolic compounds and flavonoids in plants result in stronger allelopathic effects on neighboring plants (Rashidi et al., 2020).

Since *T. subulata* leaves contain medicinal properties (Silvestre et al., 2013), this study was carried out bioassay experiment to evaluate the allelopathic potential of *T. subulata* leaves by using different solvents on selected bioassay species in laboratory. In this experiment, methanol, hexane, ethyl acetate, and distilled water were used as solvent for extraction to obtain polar and non-polar bioactive allelochemicals. Understanding different effects of solvent extracts on bioassay species can provide important insights into the future herbicide recommendation.

Material and Methods

Preparation of donor plant materials and molecular identification

Turnera subulata Sm. was used as donor plant to evaluate the possible allelopathic potential on the growth of three bioassay species. This research used yellow and off-white *T. subulata* as donor plants that grown in the Herbal Garden at Universiti Sultan Zainal Abidin (05° 45′ 10.1 N; 102° 37′ 41.9 E).

For molecular identification, the total genomic DNA from the sample was extracted using the CTAB-based method out-

Fig. 1a. Yellow *Turnera*

Fig. 1b. Off-white *Turnera*

lined by Mohd Fahmi et al. (2010) with a slight modification. The amplification process was done using the internal transcribed spacer (ITS) region adopted from ITS2 primer (Chen et al., 2010). Thermal cycling conditions for PCR Analysis were performed as in Table 1. The PCR condition used was based on Thermo Scientific™ DreamTaq™ PCR Master Mix (2X), a ready-to-use solution containing DreamTaq DNA polymerase, optimized DreamTaq buffer, $MgCl_2$ and dNTPs. The amplified product was screened using electrophoresis gel. The amplified PCR product was then sent for sequencing.

For leaf preparation procedure, the leaves were harvested, cleaned with tap water several times, and oven dried for 24 hours at 60°C. The leaves were kept at room temperature (28 ± 2°C) before use.

Table 1. Thermal cycling conditions for PCR Analysis

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension*	72	1 min	
Final extension	72	5-15 min	1

* The recommended extension step is 1 min for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb

Preparation of receiver plant materials

Brassica chinensis (mustard), *Oryza sativa* (L.) var *sativa* (weedy rice), and *Triticum aestivum* L. (wheatgrass) were the receiver plant species in this research. Seeds of mustard were purchased from Leckat Corporation Sdn Bhd and wheatgrass was purchased from WHT Wellgrow Seeds. Meanwhile, weedy rice seeds were collected from a paddy field in Kawasan Pembangunan Pertanian Bersepadu Terengganu Utara (IADA KETARA). *B. chinensis* seeds were chosen as a dicot bioassay species due to their rapid germination rate and high sensitivity. Hence, they are also widely used in previous research. Meanwhile, *T. aestivum* seeds act as monocot-tested bioassay species and *O. sativa* (L.) var. *sativa* seeds act as weed bioassay species. The seeds were surface sterilized with 0.1% (w/v) sodium hypochlorite for 3 minutes before being washed under running tap water, followed by distilled water (El Ayeb et al., 2013).

Extract preparation of donor plants

Four different solvents, hexane, ethyl acetate, methanol, and water in a ratio of 80:20 (v/v), were used to extract the leaves of *T. subulata*. 10 g of dried yellow and off-white *T. subulata* leaves were each soaked in 100 ml of the respective solvent for 72 hours at 4°C to prepare a 100 mg/ml stock solution. The solution was then centrifuged for 20 minutes at 3500 rpm and filtered through filter paper. The resulting filtrate was evaporated to dryness under a vacuum at 40°C using a rotary evaporator to remove the solvent. The extracts were then diluted with sterile distilled water to obtain concentrations of 0.1, 1.0, 10, 50, and 100 mg/ml (Motmainna et al., 2021). These extracts were stored at 4°C until they were needed for further use.

Laboratory bioassay

About thirty seeds each of weedy rice, wheatgrass, and mustard were pre-germinated for 24 hours until a root of 1mm emerged. Then, five uniform germinated seeds were sown on sterile 60 mm petri dishes lined with filter paper (Whatman No.1). Five mL of each type of extract (aqueous, methanol, ethyl acetate, and hexane) at different concentrations (0.1, 1.0, 10, 50, and 100 g/L) was prepared and applied to each petri dish separately (Aslani et al., 2015). All the petri dishes were placed in a growth chamber with the following conditions: 12 hours light, 12 hours dark, temperature: 25 ± 2°*C*, humidity: 60%, and light intensity: 743.2 FC.

After seven days of treatment, the root and shoot lengths of the bioassay species were measured, and the inhibitory effects

on root and shoot lengths were calculated. The calculation for the inhibitory effect was as follows (Kordali et al., 2009):

$$I = 100\ (C - A)/C,$$

where "I" is the inhibition percentage (%), "C" is the mean length of the root and shoot of the control, and "A" is the mean length of the root and shoot of the extracts.

Determination of total phenolic content and total flavonoid content

The Follin-Ciocalteau assay was used to determine the total phenolic content (TPC) of the samples (Singleton & Rossi, 1965). To begin, 1.5 ml of 10-fold diluted Folin-Ciocalteau reagent was mixed with 0.2 ml of diluted extract. After 5 minutes at room temperature, 1.5 ml of sodium carbonate (6%) was added and vortexed. The mixture was incubated at room temperature for 90 minutes before measuring absorbance with a spectrophotometer at 725 nm against a blank. Based on the standard curve from the gallic acid standard, the results were expressed as gallic acid equivalent (mg GAE/ g).

The Total Flavonoid Content (TFC) was assess using an aluminium chloride colorimetric assay with quercetin as the standard (Liu et al., 2008). Briefly, 0.2 ml of diluted extract was mixed with 0.2 ml of 0.5% sodium nitrate before incubation for 5 min. The mixture was then given 0.2 ml of 10% aluminium chloride and thoroughly mixed. After 6 minutes, 2 ml of 1M sodium hydroxide was added to the mixture. The mixture was made up to 5 ml with 80% methanol and thoroughly mixed. A spectrophotometer was used to measure the absorbance of the mixture at 510 nm against blank.

Statistical analysis

All experiments were conducted in a Completely Randomized Design (CRD) and shoot, and root length data were subjected to One-way Analysis of Variance (ANOVA) using Minitab Statistical Software. Separation of treatment means from the control at 0.05 probability level was conducted using Tukey test.

Results

Molecular identification of T. subulata

PCR technique was used to amplify the DNA template with forward and reverse ITS2 primers. Internal Transcripted Spacer 2 (ITS2) (5'CGATGAAGAACGCAGCGAAATGCGAT-3') is the primer used in this process. As outlined by (Chen et al., 2010), the ITS2 primer is suitable to identify DNA in medicinal plants. Table 2 indicated the NCBI result of *Turnera* species. Based on the BLAST data, the off-white and yellow *Turnera* is the species of *Turnera subulata* Sm. with a similarity percentage of 99.65% and 98.59% respectively.

Allelopathic effect of off-white and yellow T. subulata leaves on selected bioassay species

The present study determined the allelopathy effect on the inhibition of shoot and root length. The results proved that the application of *T.subulata* leaf extracts significantly diminished the shoot and root length of the targeted crops and weed studied, in which the magnitude of inhibition increased with the increase in extract concentration compared to the controls.

Data in Table 3 indicated that the shoot length of all bioassay species was significantly inhibited in their response to all extracts tested compared to the control. The heat map with cluster dendrogram showed that shoot inhibition towards bioassays species was separated into three major groups (Figure 2). The highest inhibition was marked at the highest concentration (100 mg/ml), up to 61.4% compared to the control, while the lowest inhibition was noted in the lowest treatment (0.1 mg/ml). Indeed, results have demonstrated a stimulating effect on the shoot growth of all bioassay species studied at lower concentrations (0.1 to 1.0 mg/ml). This phenomenon is known as the hormesis effect (Hadacek et al., 2011).

It was noted that the shoot inhibition on mustard is prominent, reaching up to 61.4% of inhibition if compared to wheatgrass and weedy rice. Additionally, the type of solvent used affects phytotoxicity; polar and non-polar solvents exhibit different levels of inhibition. At 100 mg/ml, non-polar solvents (hexane) only inhibited shoot growth by up to 13% shoot inhibition. For instance, all bioassay species exhibit negative inhibition except for weedy rice in aqueous extract at 0.1 mg/ml.

Simultaneously, the research also examined on the root length of tested species. Results in Figure 3 and Table 4 revealed that off-white and yellow *Turnera* extract significantly inhibited the root length of all bioassay species compared to the control. In contrast to the shoots, roots exhibit a higher level of inhibition at 100 mg/mL, ranging from 70 to 91% (Figure 3). A marked reduction was noted, the highest in mustard of off-white *Turnera* aqueous extract

Table 2. NCBI result of *Turnera subulata* Sm.

Plant species	Species identified	Accession number	Percentage identification (%)
Off-white *Turnera*	*Turnera subulata*	AY973356.1	99.65
Yellow *Turnera*		JQ723371.1	98.59

Table 3. Allelopathic effects of different concentrations of *T. subulata* leaf extract on the shoot length of mustard, wheat grass, and weedy rice (mean ± standard error)

Solvents	Bioassay sp.	Treatment (mg/ml)	Shoot Length	
			Yellow *Turnera*	Off-White *Turnera*
Aqueous	Mustard	0	2.41 ± 0.0424^{b}	2.41 ± 0.0424^{a}
		0.1	2.66 ± 0.0412^{a}	2.67 ± 0.0737^{a}
		1	2.63 ± 0.0832^{ab}	2.58 ± 0.103^{a}
		10	1.93 ± 0.0674^{c}	1.84 ± 0.0550^{b}
		50	1.84 ± 0.0638^{c}	1.71 ± 0.0733^{b}
		100	1.28 ± 0.0380^{d}	0.93 ± 0.0444^{c}
	Weedy Rice	0	7.3 ± 0.0697^{a}	7.3 ± 0.0697^{a}
		0.1	6.91 ± 0.0643^{b}	6.76 ± 0.0349^{b}
		1	6.91 ± 0.0665^{b}	6.49 ± 0.0467^{b}
		10	6.48 ± 0.108^{c}	6.04 ± 0.0476^{c}
		50	5.11 ± 0.0881^{d}	4.87 ± 0.115^{d}
		100	3.51 ± 0.0714^{e}	3.51 ± 0.0714^{e}
	Wheat-grass	0	4.7 ± 0.0594^{ab}	4.7 ± 0.0594^{b}
		0.1	4.99 ± 0.0473^{a}	4.97 ± 0.0386^{a}
		1	4.62 ± 0.0712^{b}	4.35 + 0.0624^{c}
		10	4.18 ± 0.110^{c}	4.29 ± 0.0923^{c}
		50	3.07 ± 0.0790^{d}	2.77 ± 0.0665^{d}
		100	2.28 ± 0.0571^{e}	2.28 ± 0.0571^{e}
Methanol	Mustard	0	2.41 ± 0.0424^{bc}	2.41 ± 0.0424^{a}
		0.1	2.89 ± 0.0559^{a}	2.43 ± 0.0753^{a}
		1	2.65 ± 0.0661^{ab}	2.35 ± 0.102^{a}
		10	2.28 ± 0.0962^{c}	2.28 ± 0.0962^{a}
		50	2.17 ± 0.0581^{d}	1.57 ± 0.0753^{b}
		100	1.46 ± 0.0486^{e}	1.46 ± 0.0486^{b}
	Weedy Rice	0	7.3 ± 0.0697^{a}	7.3 ± 0.0697^{a}
		0.1	7.35 ± 0.0631^{a}	7.53 ± 0.115^{a}
		1	7.56 ± 0.0888^{a}	6.85 ± 0.0689^{b}
		10	7.55 ± 0.142^{a}	6.23 ± 0.0630^{c}
		50	6.3 ± 0.0900^{b}	5.1 ± 0.0543^{d}
		100	3.9 ± 0.0768^{c}	3.9 ± 0.0768^{e}
	Wheat-grass	0	4.7 ± 0.0594^{a}	4.7 ± 0.0594^{a}
		0.1	5.03 ± 0.0573^{a}	5.16 ± 0.0306^{b}
		1	4.79 ± 0.0771^{a}	4.7 ± 0.0276^{b}
		10	4.14 ± 0.0985^{b}	4.59 ± 0.0624^{b}
		50	3.82 ± 0.105^{b}	3.48 ± 0.0500^{c}
		100	3.27 ± 0.0978^{c}	3.27 ± 0.0978^{c}
Ethyl acetate	Mustard	0	2.41 ± 0.0424^{b}	2.41 ± 0.0424^{b}
		0.1	2.87 ± 0.0575^{a}	2.79 ± 0.0584^{a}
		1	2.86 ± 0.0773^{a}	2.86 ± 0.0773^{a}
		10	2.64 ± 0.0877^{ab}	2.67 ± 0.0463^{a}
		50	2.47 ± 0.0441^{b}	2.31 ± 0.0576^{b}
		100	2.04 ± 0.0486^{c}	2.04 ± 0.0486^{c}
	Weedy Rice	0	7.3 ± 0.0697^{b}	7.3 ± 0.0697^{bc}
		0.1	7.69 ± 0.102^{ab}	7.82 ± 0.176^{a}
		1	7.67 ± 0.0583^{ab}	7.67 ± 0.0583^{ab}
		10	7.86 ± 0.139^{a}	7.43 ± 0.0802^{abc}
		50	7.37 ± 0.0672^{b}	7.22 ± 0.0895^{c}
		100	6.35 ± 0.103^{c}	6.35 ± 0.103^{d}
	Wheat-grass	0	4.7 ± 0.0594^{b}	4.7 ± 0.0594^{b}
		0.1	4.97 ± 0.0575^{a}	5.23 ± 0.0643^{a}
		1	4.7 ± 0.0276^{b}	4.7 ± 0.0276^{b}
		10	4.55 ± 0.0822^{b}	4.72 ± 0.0480^{b}
		50	4.53 ± 0.0784^{b}	4.22 ± 0.0571^{c}
		100	3.69 ± 0.0345^{c}	3.69 ± 0.0345^{d}
Hexane	Mustard	0	2.42 ± 0.0447^{b}	2.41 ± 0.0424^{c}
		0.1	2.9 ± 0.0535^{a}	2.88 ± 0.0449^{a}
		1	2.85 ± 0.0533^{a}	2.85 ± 0.0533^{ab}
		10	2.81 ± 0.0636^{a}	2.69 ± 0.0456^{b}
		50	2.37 ± 0.0463^{b}	2.41 ± 0.0511^{c}
		100	2.09 ± 0.0401^{c}	2.09 ± 0.0401^{d}
	Weedy Rice	0	7.3 ± 0.0697^{c}	7.3 ± 0.0697^{b}
		0.1	7.67 ± 0.101^{ab}	7.71 ± 0.0980^{a}
		1	7.77 ± 0.0688^{a}	7.77 ± 0.0688^{a}
		10	7.62 ± 0.0972^{abc}	7.53 ± 0.140^{ab}
		50	7.4 ± 0.0743^{bc}	7.17 ± 0.0733^{b}
		100	6.1 ± 0.0926^{d}	6.1 ± 0.0926^{c}
	Wheat-grass	0	4.7 ± 0.0594^{c}	4.7 ± 0.0594^{bc}
		0.1	5.01 ± 0.0589^{ab}	5.55 ± 0.123^{a}
		1	5.03 ± 0.0613^{a}	5.03 ± 0.0613^{b}
		10	4.77 ± 0.0636^{bc}	4.78 ± 0.0829^{b}
		50	4.44 ± 0.0646^{d}	4.19 ± 0.0774^{d}
		100	4.44 ± 0.0600^{d}	4.44 ± 0.0600^{cd}

Note: Means within the columns followed by the same alphabet were not significantly different ($p>0.05$) according to DMRT. The values given in the table were the mean of all the parameters over that of the control

(91.67%), followed by mustard of yellow *Turnera* aqueous extract (88.96%) and mustard of methanolic off-white *Turnera* extract (77.92%). There was no clear separation between yellow and off-white *Turnera* extract, and both showed high inhibition in mustard at 100 mg/ml.

Meanwhile, the root inhibition in monocot bioassay species is still low compared to dicotyledon (mustard). The extent of root inhibition in monocots was the highest in the aqueous extract of yellow *Turnera* (wheat; 30-50%, weedy rice; 60-76%). Among the treatment concentrations, the lowest concentration still exhibits a hormesis effect, inducing the root growth of bioassays species. The results indicated that *T. subulata* leaves significantly suppressed ($p<0.05$) the plant growth when exposed to increasing concentrations and

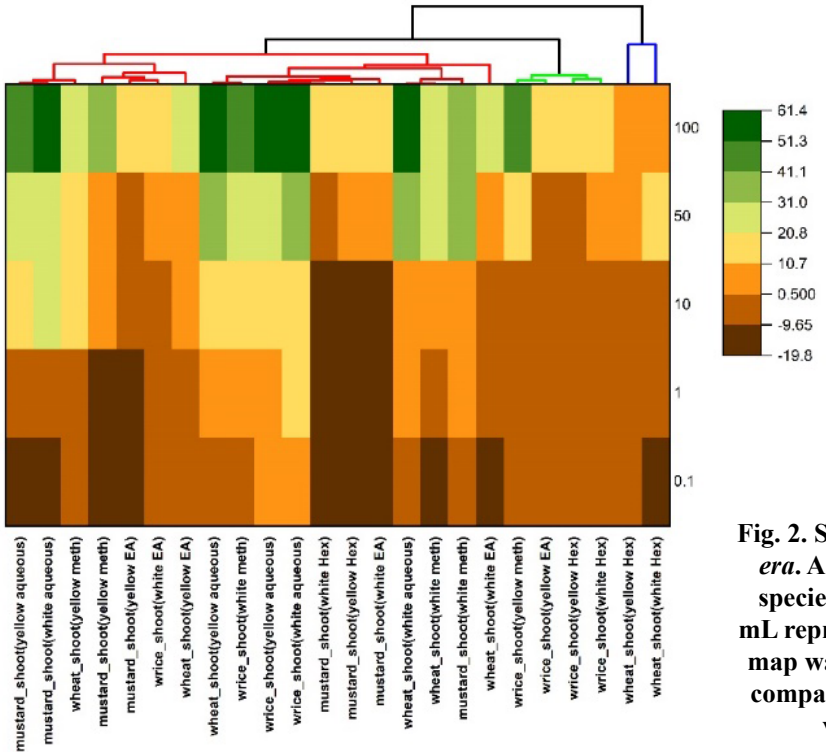

Fig. 2. Shoot inhibition of yellow and off-white *Turnera*. A Heat map with a dendrogram of bioassays species and treatment. 0.1, 1, 10, 50, and 100 mg/mL represents the treatment concentration and heat map was generated based on inhibition percentage compared to control. Green represents the highest value and brown for the lowest value.

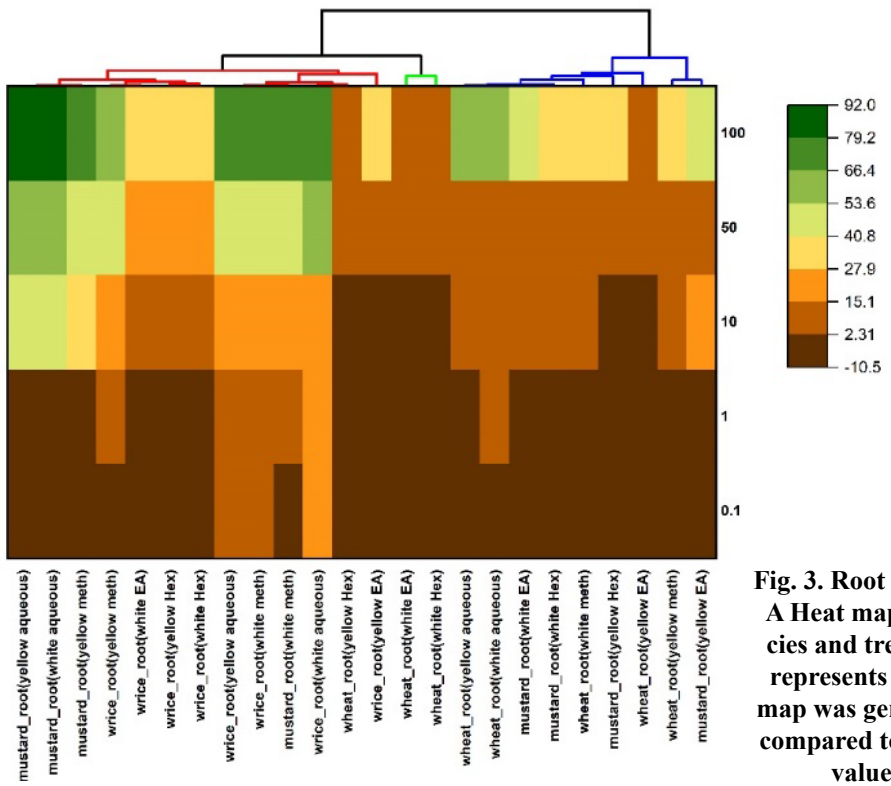

Fig. 3. Root inhibition of yellow and white *Turnera*. A Heat map with a dendrogram of bioassays species and treatment. 0.1, 1, 10, 50, and 100 mg/mL represents the treatment concentration and heat map was generated based on inhibition percentage compared to control. Green represents the highest value and brown for the lowest value.

Table 4. Allelopathic effects of different concentrations of *T. subulata* leaf extract on the root length of mustard, wheat grass, and weedy rice (mean ± standard error)

Solvents	Bioassay sp.	Treatment (mg/ml)	Root Length	
			Yellow *Turnera*	Off-White T*urnera*
Distilled water	Mustard	0	2.96 ± 0.0363^{a}	2.96 ± 0.0363^{a}
		0.1	2.94 ± 0.0388^{a}	2.9 ± 0.0468^{a}
		1	2.9 ± 0.0378^{a}	2.89 ± 0.0330^{a}
		10	1.74 ± 0.0646^{b}	1.74 ± 0.0600^{b}
		50	1.19 ± 0.0636^{c}	1.07 ± 0.0597^{c}
		100	0.33 ± 0.0330^{d}	0.25 ± 0.0274^{d}
	Weedy Rice	0	8.7 ± 0.176^{a}	8.7 ± 0.176^{a}
		0.1	8.03 ± 0.124^{b}	7.23 ± 0.0714^{b}
		1	7.57 ± 0.0923^{b}	7.11 ± 0.0628^{b}
		10	6.35 ± 0.0945^{c}	6.34 ± 0.0616^{c}
		50	4.25 ± 0.0839^{b}	3.91 ± 0.101^{d}
		100	2.31 ± 0.0556^{c}	2.01 ± 0.0433^{e}
	Wheatgrass	0	4.75 ± 0.0608^{a}	4.75 ± 0.0608^{ab}
		0.1	4.97 ± 0.0651^{a}	4.93 ± 0.0523^{a}
		1	4.73 ± 0.0693^{a}	4.54 ± 0.0638^{b}
		10	4.23 ± 0.0785^{b}	4.2 ± 0.0717^{c}
		50	4.31 ± 0.0861^{b}	4.23 ± 0.0877^{c}
		100	2.11 ± 0.0551^{c}	2.11 ± 0.0551^{d}
Methanol	Mustard	0	2.96 ± 0.0424^{a}	2.96 ± 0.0363^{a}
		0.1	2.99 ± 0.0559^{a}	2.97 ± 0.0433^{a}
		1	2.98 ± 0.0661^{a}	2.87 ± 0.0441^{a}
		10	1.94 ± 0.0962^{b}	2.26 ± 0.0872^{b}
		50	1.53 ± 0.0581^{c}	1.38 ± 0.0823^{c}
		100	0.67 ± 0.0486^{d}	0.67 ± 0.0435^{d}
	Weedy Rice	0	8.7 ± 0.176^{a}	8.7 ± 0.176^{a}
		0.1	8.75 ± 0.113^{a}	8.7 ± 0.0892^{b}
		1	7.86 ± 0.106^{b}	7.41 ± 0.0646^{b}
		10	6.37 ± 0.110^{c}	6.4 ± 0.413^{c}
		50	4.72 ± 0.0603^{d}	4.25 ± 0.0584^{d}
		100	3.35 ± 0.0774^{e}	2.56 ± 0.0904^{e}
	Wheatgrass	0	4.75 ± 0.0608^{ab}	2.96 ± 0.0608^{a}
		0.1	4.96 ± 0.0524^{a}	2.97 ± 0.0374^{a}
		1	4.88 ± 0.0626^{ab}	2.87 ± 0.0388^{a}
		10	4.21 ± 0.0798^{c}	2.26 ± 0.0733^{b}
		50	4.62 ± 0.0712^{b}	1.38 ± 0.0530^{c}
		100	3 ± 0.0535^{d}	0.67 ± 0.0535^{d}
Ethyl acetate	Mustard	0	2.96 ± 0.0363^{a}	2.96 ± 0.0363^{b}
		0.1	2.96 ± 0.0533^{a}	3.21 ± 0.0483^{a}
		1	3.04 ± 0.0306^{a}	3.04 ± 0.0306^{a}
		10	2.45 ± 0.0689^{b}	2.89 ± 0.0565^{b}
		50	2.68 ± 0.0972^{b}	2.87 ± 0.0613^{b}
		100	1.73 ± 0.0651^{c}	1.73 ± 0.0651^{c}
	Weedy Rice	0	8.7 ± 0.176^{b}	8.7 ± 0.0608^{b}
		0.1	8.85 ± 0.136^{ab}	9.59 ± 0.0396^{a}
		1	9.25 ± 0.122^{a}	9.25 ± 0.0689^{a}
		10	8.5 ± 0.117^{b}	8.19 ± 0.0504^{b}
		50	7.47 ± 0.0849^{c}	7.07 ± 0.0591^{c}
		100	6.26 ± 0.125^{d}	6.26 ± 0.0679^{d}
	Wheatgrass	0	4.75 ± 0.0608^{ab}	4.75 ± 0.0608^{bc}
		0.1	4.93 ± 0.0679^{a}	5.21 ± 0.0396^{a}
		1	4.85 ± 0.0689^{ab}	4.85 ± 0.0689^{b}
		10	4.79 ± 0.0628^{ab}	4.73 ± 0.0504^{bc}
		50	4.62 ± 0.0562^{b}	4.53 ± 0.0591^{c}
		100	4.07 ± 0.0679^{c}	4.07 ± 0.0679^{d}
Hexane	Mustard	0	2.96 ± 0.0363^{a}	2.96 ± 0.0363^{ab}
		0.1	3.02 ± 0.0656^{a}	3.19 ± 0.0749^{a}
		1	2.97 ± 0.0384^{a}	2.97 ± 0.0384^{ab}
		10	2.92 ± 0.0619^{a}	2.87 ± 0.0607^{b}
		50	2.85 ± 0.0559^{a}	2.89 ± 0.0565^{b}
		100	1.99 ± 0.0733^{b}	1.99 ± 0.0733^{c}
	Weedy Rice	0	8.7 ± 0.176^{a}	8.7 ± 0.176^{a}
		0.1	8.94 ± 0.159^{a}	8.96 ± 0.116^{a}
		1	8.61 ± 0.0515^{a}	8.61 ± 0.0515^{a}
		10	7.64 ± 0.0985^{b}	7.83 ± 0.0853^{b}
		50	7.08 ± 0.0698^{c}	6.96 ± 0.0809^{c}
		100	5.95 ± 0.100^{d}	5.95 ± 0.100^{d}
	Wheatgrass	0	4.75 ± 0.0608^{ab}	4.75 ± 0.0608^{bc}
		0.1	4.95 ± 0.0761^{a}	5.17 ± 0.0853^{a}
		1	4.89 ± 0.0654^{a}	4.89 ± 0.0654^{ab}
		10	4.81 ± 0.0581^{ab}	4.69 ± 0.0533^{bc}
		50	4.53 ± 0.0686^{bc}	4.52 ± 0.0480^{cd}
		100	4.28 ± 0.105^{c}	4.28 ± 0.105^{d}

Note: Means within the columns followed by the same alphabet were not significantly different (p>0.05) according to DMRT. The values given in the table were the mean of all the parameters over that of the control.

different solvents. Thus, it was proven that the tested species were more tolerant in the highest concentration as the growth will suppress more than 70% of polar solvents.

Total phenolic content and total flavonoid content of T. subulata leaf extract

A standard curve was generated by plotting the absorbance values of gallic acid and quercetin against different concentrations to determine the total phenolic content (TPC) and total flavonoid content (TFC), respectively. The total TPC and TFC of off-white and yellow *Turnera* were obtained based on calculation from the standard calibration curve and recorded in Table 5. The total phenolic content was found to be higher in the off-white *Turnera* than in the yellow *Turnera*, with values of 18.09 (gaE/g) and 16.21 (gaE/g), respectively. The results of total flavonoid content showed a similar

Table 5. Evaluation of Total Flavonoid content and Total Phenolic content of *Turnera subulata* leaves extract

No.	Sample name	Flavonoid content (ug/ml)	Phenolic content (gaE/g)
1	Off-white *Turnera*	12.5	18.09
2	Yellow *Turnera*	11.3	16.21

pattern to the results of TPC, with the Off-white *Turnera* has a higher total flavonoid content than yellow *Turnera*, with results of 12.5 μg/ml and 11.3 μg/ml, respectively.

Discussion

Allelopathic effects were first exerted by the polar solvent, specifically an aqueous extract at different concentrations. This was followed by methanol, ethyl acetate, and hexane. Data presented proved that allelochemicals contained in leaves of *T. subulata* had comparatively greater solubility in aqueous extract. The significant decrease in the aqueous extract is attributed to the presence of a water-soluble phenolic compound in the species study (Heidarzade et al., 2012). Moreover, the extraction of plant metabolites is more efficient in polar solvents than in non-polar solvents, as reflected by Li & Jin (2010). The present result was supported by Aslani et al. (2016), who examined that the water extract of *Tinospora tuberculata* Sm. reduced by 81% of barnyard grass radicle development.

According to the results, the root inhibition was the most apparent compared to the shoot in all tested species of all solvents. The phytochemical substance of *T. subulata* extracts was highly suppressive, especially in roots that were inhibited even at the lowest concentration. The root is the first organ that comes in contact with the extract and thus expresses higher inhibition than shoots (Islam & Kato-Noguchi, 2013). Another finding by Nurul Ain et al. (2017) and Wan Abdul Halim et al. (2022) stated that *T. subulata* had a strong allelopathic effect on the root length of species tested by more than 50%, whereas similar result had been reported by Ishak et al. (2016) that the inhibitory effect on the shoot length was less than 50%.

The phytochemical released from the extract may interrupt the plant metabolic pathway by disrupting the plant defense mechanism, resulting in cell damage which leads to root inhibition (Azlan et al., 2022; Anese et al., 2015; Nishida et al., 2005; Cai & Mu, 2012). Besides, the root has many permeable tissues and a thin cuticle layer, which allows the transportation of phytochemical compounds more than the shoot (Islam et al., 2019; Grana et al., 2013). The explanation was supported by Cai & Mu (2012), who studied the treatment of soybean with aqueous leaf extracts from *Datura stramonium*. It was found that primary root reduction was seen in higher concentrations of the extracts.

The trend of the present result showed that 100 mg/ml possesses the greatest inhibitory effect as compared to others. It was parallel with the observation that Kyaw & Kato-Noguchi (2020) and Bari & Kato-Noguchi (2019) state that the inhibitory activity increased with the increasing extract concentration. Motmainna et al. (2021) examined that the reduction in root elongation was noted the highest in 100 mg/ml, which was above 93 to 100%. As Golubinova & Ilieva (2014) stated, lower concentrations of extracts could attract fewer allelochemicals so that less reduction could be recorded in the lowest concentration. If an allelopathic approach is to be utilized in the field, it is necessary to identify the concentration at which a particular response occurs.

Besides, the highest inhibitory effect was observed in dicot plants (mustard), even in the lowest concentration compared to monocot. Similarly, Bari et al. (2019) and Chen et al. (2017) noted that highest inhibitory effect was observed in dicot plants. Indeed, such ability was due to the allelopathic activity, which can vary among species according to the solvent used, extract concentration, and allelochemical presented in the species (Yoshimura et al., 2011). Besides, the difference in inhibition effects could be attributed to plant cell structure and elongation (Cheng & Cheng, 2015).

The screening of the total phenolic and total flavonoid compounds in off-white and yellow *Turnera* leaf have been identified as being composed of phenolic and flavonoid compounds, which have previously been reported to have herbicidal activity. The result was found to be higher in off-white *Turnera* for both TPC and TFC, with value 18.09 gaE/g and 12.5 ug/ml. The value obtained was in range with the study of Saravanan et al. (2020).

As outlined by (Sharma et al., 2019; Lin et al., 2016), phenolic compounds could hinder plant growth by binding to and inhibiting enzymes involved in cell division and elongation. Additionally, they can affect the uptake and utilization of important nutrients such as nitrogen and phosphorus, which are crucial for plant growth. These compounds can alter root and shoot development of bioassay species by disrupting the phenylpropanoid pathway. Meanwhile, the growth-inhibiting effect of flavonoids can alter the hormonal balance in plants, specifically by affecting levels of auxins and cytokinins, which reduced root and shoot growth of bioassay species, respectively. It is believed that the reduction in root and shoot growth of the bioassay species studied was a result of the presence of phenolic and flavonoid compounds in the extracts.

Therefore, *T.subulata* has the potential to be a valuable plant for use as a component in a bioherbicide that helps to

control the growth of undesirable weeds. However, the results should be validated with greenhouse experiments. A study by Sahrir et al. (2023) found that results from laboratory allelopathy experiments should be validated through further testing in the greenhouse or field. This trend of incorporating greenhouse and field trials into allelopathy research highlights the significance of these trials as complementary data to laboratory bioassay studies.

Conclusions

The results of this study emphasize the significance of investigating natural and environmentally friendly methods for weed control. The off-white and yellow leaves of *Turnera subulata Sm.*have been found to have herbicidal properties when tested on selected bioassay species over aqueous, methanol, hexane, and ethyl acetate extract. The strength of the herbicidal activity was dependent on the species tested, the concentration of the extract, and the solvent used for extraction. The aqueous extract showed the highest level of root and shoot inhibition among all solvents tested, with roots being more susceptible to inhibition than shoots. This suggests that *T. subulata* could be a promising plant species for reducing weed growth in agriculture field through allelopathy. Further research, including validation under greenhouse conditions, is necessary to provide practical recommendations for farmers. Additionally, future studies should aim to identify and profile the specific allelopathic compounds in *T. subulata* through Liquid Chromatography-Mass Spectrometry analysis.

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