RESEARCH ARTICLE

Allelopathic Effects of Invasive Alien Plant *Tithonia diversifolia* (Hemsl.) A. Gray on *Pisum sativum* L.

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Abstract

The essential factors for the success of invasive alien plants are considered to be the presence of allelochemicals, which are considered "novel weapons". The allelochemicals released by invasive plants interact differently with each other, especially native and endemic diversity, and also serve as a biological defense against plant diseases and pathogens. To this end, *Tithonia diversifolia* (Hemsl.) A. Gray, an emerging invasive alien plant in the northeastern (NE) Himalayas, is scantly investigated in terms of exhibiting allelopathy, despite being an aggressive weed in Mizoram, NE India. Especially, their effects on widely edible crops need to be studied because of their inextricable linkage with food productivity and rural livelihood. Thus, the present investigation was carried out on the allelopathic effects of *T. diversifolia*, based on the physical parameters such as SH, RL, SL, B, GPe, GPo, GI, GRI and VI on the selected common crop *Pisum sativum* L. through a pot bioassay experiment. In this respect, two highly invaded sites of *T. diversifolia* were selected for the collection of soil samples based on the variable disturbance factors. The present result concluded that *T. diversifolia* has strong stimulatory allelopathic effects on the growth parameters of *P. sativum* at the initial stage of the seed germination; however, it tends to normalize at the later stage of germination. The infested soil of *T. diversifolia* was observed to be suitable for growing food crops, although it may take longer to germinate due to the allelopathic effect of *T. diversifolia*. Nevertheless, wise utilization of the leaf leachates may enhance soil fertility for better production of food crops and facilitate sustainable agriculture.

Keywords: Novel weapon hypothesis, Secondary metabolites, *Pisum sativum*, Allelopathy, Seed germination.

Highlights

- The novel allelopathic compounds act as a weapon to inhibit and stimulate the growth/development of native plants or edible food crops.
- *T. diversifolia* is enriched with allelochemicals, is an emerging invasive alien plant in NE India, but with limited studies on allelopathy.
- To know the allelopathic effect of *T. diversifolia* on a crop (*Pisum sativum* L.), a pot bioassay was conducted.
- *T. diversifolia* showed strong stimulatory allelopathic effects at the initial stage on the growth of P. sativum.

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Introduction

The lack of natural enemies, i.e., "enemy release hypothesis," and the introduction of novel allelopathic compounds give invasive alien plants a competitive advantage to disrupt native ecosystems and crops (Blumenthal *et al*., 2003; Lenda *et al*., 2023). The disturbed environments, which underwent forest fire, burning through shifting cultivation and waterlogged or flooded, made agricultural ecosystems as well as urbanized habitats more susceptible to invasion (Callaway and Ridenour, 2004; Brasseur *et al.,* 2024). Transportation infrastructures (such as roads or railways) also facilitate the abrupt spread of invasive alien plants (Callaway and Ridenour, 2004; Syngkli *et al*., 2025). The biological properties highly affected the successful spread of invasive alien plant species (Rai and Singh, 2020). To this end, the allelochemical compounds in invasive plants in fact act as novel weapons (linked with "Novel weapon hypothesis") to suppress native indigenous plants and edible crops (Callaway and Ridenour, 2004; Syngkli and Rai, 2024).

One of the most essential factors in the success of invasive alien plants is considered to be the presence of allelochemicals, which are considered novel weapons against native plants and food crops (Chengxu *et al.,* 2011; Syed *et al*., 2021). These allelochemicals are the secondary metabolites produced in the environment by organisms or a plant invader, which can inhibit or enhance the growth of other nearby native plants or food crops (Syngkli *et al.,* 2022a) as *T. diversifolia* is known to have secondary metabolites such as sesquiterpenoids, terpenoids, flavonoids, tannins and saponins (Zhou *et al*., 2013; Noguchi, 2020 and Omolola, 2020), their allelopathic effects need to be

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pragmatically evaluated. They enable them to increase their population in the newly invaded environment by suppressing native plants (Chengxu *et al*., 2011; Meiners *et al*., 2012). This process of interaction is known as allelopathy (Perveen *et al*., 2020), which is still inadequately explored in the context of *T. diversifolia*.

Allelopathy influences plants' invasiveness and is associated with the naturalization and establishment of invasive species in non-native novel introduced habitat ranges (Noguchi, 2020). They are produced as one or more secondary metabolites that affect other organisms within the same community in terms of germination, growth, survival, and reproduction (Manivel *et al.,* 2023). Alllelopathy is broadly classified into direct and indirect allelopathy based on the involvement of intermediate organisms (Manivel *et al.,* 2023). There are substantial differences in these phytotoxins' chemical makeup, mode of action, and effects on plants, which could be either inhibitory or stimulatory (Kaur, 2012; Syngkli *et al.,* 2022a). Allelochemicals act as an important component of both managed and natural ecosystems; allelochemicals released by invasive alien plants also offer biological defense against plant diseases and pathogens, thereby facilitating their invasive spread (Syngkli *et al.,* 2022b).

T. diversifolia was reported to influence the germination, growth and chlorophyll accumulation of tomato, maize and *Amaranthus cruentus* (Otunsanya *et al.,* 2007; Otunsanya *et al.,* 2015). Past studies noted that *T. diversifolia* also exhibits stimulatory effects on the vegetative growth of cowpea and maize (Aladejimokun *et al*., 2014). Further, Adesina (2013) also reported that soil collected from *T. diversifolia* infested fields suppressed the emergence of *Ancanthospermum hispidium*, *Bidens pilosa*, *Euphorbia heterophylla*, *Panicum masimum*, and *Pennisetum polysachion* ascribed to its allelopathic effects. The allelochemical compounds may accumulate as a result of the exudation from living plant tissues of *T. diversifolia* or as a result of the plant residues or leaf leachates breaking down in the soil to release allelochemicals (Noguchi, 2020). As the influences are seen in both forestry, agroforestry, and agriculture sectors, the chemical ecology studies are desirable to examine the effects of released allelochemical compounds on the growth and development of edible food crops (Syngkli *et al.,* 2022b).

The present article selected *T. diversifolia*, an emerging invasive alien plant in the northeast (NE) Himalayas, which is scantily investigated in terms of exhibiting allelopathy, despite the widely accepted status of an aggressive weed in Mizoram, NE India (Vanlalruati and Rai, 2021). Further, the rationale behind selecting *T. diversifolia* is ascribed to its adverse socio-economic and environmental effects at the national and global scale (Rai and Singh, 2021; Rai *et al.,* 2023). Further, it belongs to the family Asteraceae with aggressive functional traits. and listed as an invasive alien plant species in Mizoram, among 163 alien plant species found in Mizoram (Sengupta and Dash, 2020; Rai and Singh, 2024). The indigenous tribal community of Mizoram widely perceives that *T. diversifolia* is harmful in the context of the environment and socio-economy; therefore, it warrants focused studies to elucidate the ecological mechanism responsible for its invasive spread in NE India, especially Mizoram (Rai and Vanlalruati, 2022). Despite its established landscape spread in NE India, the information on *T. diversifolia* is very limited as compared to other invasive alien plants such as *Parthenium hysterophorus* L., *Chromolaena odorata* L., *Mikania micrantha* Kunth, and *Ageratum conyzoides* L. (Rai, 2012, 2015; Sakachep and Rai, 2025). Especially, allelopathic effects of *T. diversifolia* on protein-rich food crops are inadequately explored through pot bioassay experiments, which necessitated the design of the present research.

In the present study, the *Pisum sativum* L. (common pea) was used as a model assay crop for investigating the allelopathic effects of *T. diversifolia* on soil (Girija and Gowri, 2008). It is one of the popular crops grown in agricultural fields as well as home gardens in Mizoram. Among the Fabaceae family, *P. sativum* is considered as second most important crop as it contains major components of dietary requirements, including protein, fat, and carbohydrates in the form of starch (Shanthakumar *et al*., 2022). Due to its short life span, i.e., 50-70 days, pea has emerged as one of the most-used plants for studying plant bioassay and ecotoxicological studies (Kim *et al*., 2022; Mobley *et al*., 2022). Past studies also validated the utility of *P. sativum* as a bioassay crop because of its rapid response to environmental contaminants (Rosner *et al*., 2020; Nikolov *et al.,* 2023). Therefore, the main objectives of this study were to assess the allelopathic impacts of *T. diversifolia* invaded soil on the growth parameters of *P. sativum*, which may have implications in maintaining crop yield of traditional agriculture systems of N.E. India.

Materials And Methods

Study area

The study was conducted in Aizawl, the capital of Mizoram, located in northeastern (NE) part of India (Fig. 1). It lies between 92°3'- 92°60' E longitude and 21°58'- 24°85' N latitude, and occupied 6.96% of the total geographical area of the state (District census handbook, 2011; Vanlalruati and Rai, 2021; Sakachep and Rai, 2021). The region underlies in an Indo Myanmar global biodiversity hotspot of extreme ecological relevance in terms of both ecological threats assessment and biodiversity conservation perspective (Rai, 2009) (Fig. 1). In present study, two highly invaded sites of *T. diversifolia* were selected for collection of soil samples in Aizawl city based on the disturbance factor *i.e.,* disturbed and moderately disturbed sites. The moderately disturbed site of the region was on the outskirts of the city and noted with partially intact natural vegetation. The dominant tree species at the moderately disturbed sites were *Pinus kesiya* Royle ex Gordon*, Artocarpus heterophyllus* Lam. *Balakata baccata* (Roxb.) Esser*, Castanopsis sp., Schima wallichii* (DC.) Korth, *Rhododendron arboreum* Sm.*, Rhus semialata* Mill.*, Ficus auriculata* Lour*,* etc. Whereas, the disturbed site was under ecological stress due to the operation of significant anthropogenic activities, such as road construction, quarrying, construction of a recreational spot, etc., and there are rapid changes in vegetation.

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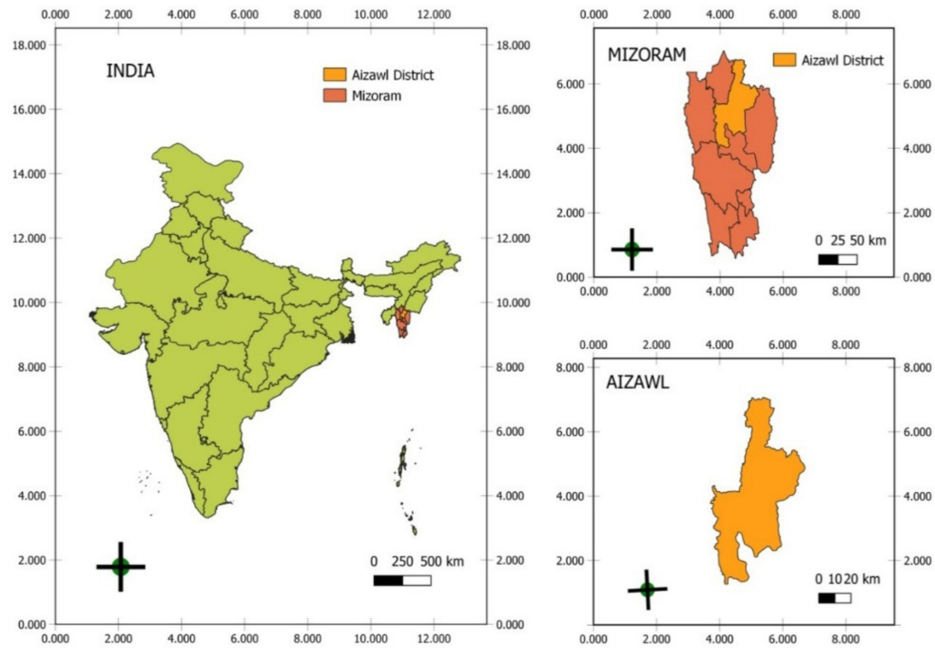

Fig 1: Map of study area located in Aizawl, Mizoram, N.E. India, located in the Indo-Myanmar global biodiversity hotspot

Lam. *Balakata baccata* (Roxb.) Esser, *Castanopsis sp., Schima wallichii* (DC.) Korth, *Rhododendron arboreum* Sm., *Rhus semialata* Mill., *Ficus auriculata* Lour, etc. Whereas, the disturbed site was under ecological stress due to the operation of significant anthropogenic activities, such as road construction, quarrying, construction of a recreational spot, etc., and there are rapid changes in vegetation.

Methods

The experiment was conducted during June-July 2023, in which the experimental pots having a 15 cm diameter and 16 cm depth were taken and filled with soil collected from disturbed and moderately disturbed sites of *T. diversifolia*; the control pot was filled with healthy forest soil. The physicochemical characteristics of soil, such as pH, soil moisture (Anderson and Ingram, 1993), soil organic carbon and soil organic matter (Walkley and Black, 1934) were analyzed with standard methods, as these soil attributes can influence the allelochemical effects of *T. diversifolia*. The rhizosphere soil collected from disturbed and moderately disturbed sites is indicated as experimental pots 1 and 2, respectively. For optimal drainage, 6 holes were perforated at the bottom of both the experimental and control pots. The most commonly grown crop, *P. sativum* was chosen due to its fast-growing plants, simultaneous germination and the seed homogeneity; thus total of thirty seeds were sown (10 seeds in each pot) on control and experimental 1and 2, with the same amount of sunlight and water. The number of seeds that germinated was noted on the third and seventh days. At various intervals, the plants in the three pots were closely inspected to detect any morphological changes. When the crop is fully grown, i.e., on the 60th day, three replicas (Sample A, B, and C) from each pot were collected. The plant seedling height (SH), root length (RL) and shoot length (SL) were measured with a ruler and fresh weight biomass (B) was measured in a laboratory electronic balance. Whereas, the following formulas were used for the calculation of Germination Percentage (GPe), Germination Potential (GPo), Germination Index (GI), Germination Rate Index (GRI) and Vigor Index (VI). The results of the experimental and control pots were analyzed and compared (Kumar *et al.,* 2011; Singh *et al.*, 2014; Syngkli *et al.*, 2022a, b).

Germination Percentage (GPe)

$$\mathbf{GPe} = \frac{\text{Number of seeds germinated on the } 7^{th} \text{ day}}{\text{Total number of seeds}} \text{ X } 100$$

Germination Potential (GPo)

$$\mathbf{GPo} = \frac{\text{Number of seeds germinated on the } 3^{rd} \text{ day}}{\text{Total number of seeds}}$$

Germination Index (GI)

GI = ΣGi/I

Where Gi is the number of germinations in I [the time after cultivation (day)].

iv) Germination Rate Index (GRI)

Table 1: Physicochemical characteristics of control and *T. diversifolia* infested soil

S. No.	*Parameters*	*Control*	*Experimental pot 1*	*Experimental pot 2*
1	pH	6.45 ± 0.23	6.42 ± 1.81	6.32 ± 0.53
2	Soil moisture content (%)	23.15 ± 0.35	17.42 ± 2.96	21.87 ± 6.82
3	Water holding capacity (%)	61.10 ± 0.01	67.27 ± 3.76	76.80 ± 1.10
4	Organic carbon content (%)	2.06 ± 0.30	0.61 ± 0.95	1.53 ± 0.50
5	Organic matter content (%)	3.55 ± 0.30	1.05 ± 0.95	2.64 ± 0.50

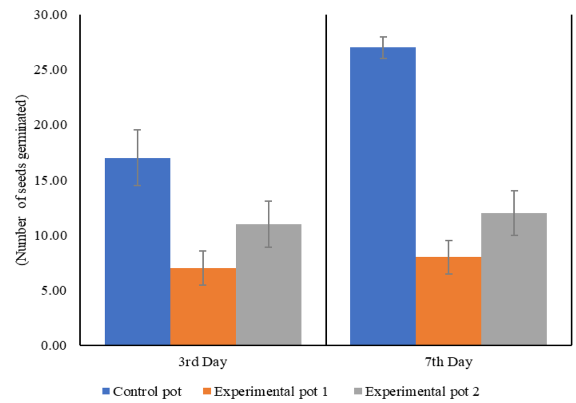

Fig. 2: Number of seeds germinated on the 3rd and 7th day Seedling height, root length, and shoot length

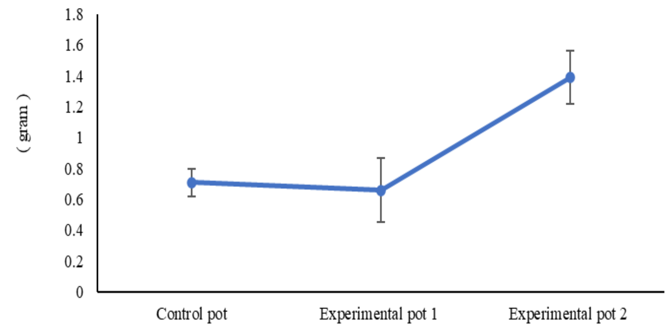

Fig 4: Seedling biomass for control and experimental pots

GRI = GPe × GI

v) Vigor Index (VI)

VI = GI × BM

Results And Discussion

Physico-chemical characteristics of soil

The physicochemical characteristics of soil are represented in Table 1. The comparative assessment of *P. sativum* growth parameters in control and *T. diversifolia* infested soil was performed as stated in Table 1.

Seeds germination

In the experimental pots, there was less germination of the *P. sativum* seeds compared to the control pot. The germination was observed to increase from the 3rd day to the 7th day in all the pots but the germination was noted lowest in experimental pot 1 followed by experimental pot 2 and control pot (Fig. 2). This showed that *T. diversifolia* invaded forest soil has least potential to grow crop at the initial stage than the disturbed soil and showed the sign of allelopathy. Allelochemical compounds released into the soil by exudates from living plant tissues or the decomposition of plant residue of invasive alien plants inhibit germination, seedling establishment, and plant growth (Bais *et al.,* 2006; Bonanomi *et al.,* 2006; Belz, 2007). Thus, *T. diversifolia* has shown an allelopathic influence on the germination and growth of *P. sativum.* Such inhibitory effects on seed germination were also reported on other plant species or edible crops such as *Tridax procumbens*, *Brassica oleracea*, *Avena sativa*, *Allium cepa*, *Solanum lycopersicum*, *Triticum aestivum* and *Amaranthus viridis* (Ademiluyi, 2013; Noguchi, 2020).

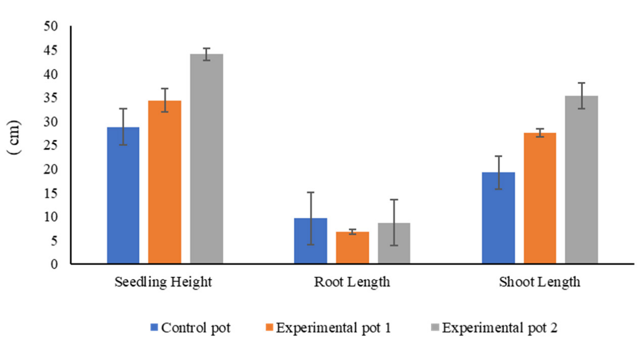

Fig 3: Seedling height, root length and shoot length of *P. sativum* on control and experimental pots

The seedling height and the shoot length were highest in experimental pot 2 followed by experimental pot 1 and control pot whereas the root length was lowest in experimental pot 1 followed by experimental pot 2 and control pot (Fig. 3). In this respect, the experiment conducted by Musyimi *et al.,* (2015) found that the shoot length of *P. sativum* was between 53– 57 cm which was even more than the shoot length found in the present experiment (19–35 cm).

In the present study, the root lengths were extremely low in all the pots when compared to the effect of *Nicotiana plumbaginifolia* rhizosphere on *P. sativum,* which was 24.07 cm in length (Mushtaq *et al.*, 2021). From the experimental results, *P. sativum* growing on rhizosphere soil of *T. diversifolia* has a mean value of 9.58 ± 2.47, 6.8 ± 0.57 and 8.73 ± 0.85 cm on control, experimental 1 and experimental 2 pots, respectively (Fig. 3). Similarly, the root length of the seedling in the control pot (18cm) revealed that allelopathic effects of *T. diversifolia* on *P. sativum* in present experiment was even stronger than its effects the germination of *Amaranthus creutusthan* where the root length in the experimental pots were noted to be about 14cm in length (Otusanya *et al.,* 2007).

Table 2: Different parameters that show the morphological changes of P. sativum grown on experimental pots and control pot

Parameters		*Control pot*	*Experimental pot 1*	*Experimental pot 2*
Germination	3rd	17	7	11
	7th	27	8	12
Seedling height (cm)		28.81 ± 3.74	34.35 ± 5.44	44.08 ± 3.52
Root Length (cm)		9.58 ± 2.47	6.8 ± 0.57	8.73 ± 0.85
Shoot Length (cm)		19.23 ± 1.28	27.55 ± 4.88	35.35 ± 2.73
Seedling Biomass (g)		0.71 ± 0.09	0.66 ± 0.21	1.39 ± 0.17
Germination percentage (%)		90 ± 10	26.67 ± 15.28	40 ± 20
Germination Potential		0.57 ± 0.25	0.23 ± 0.15	0.37 ± 0.21
Germination Index		1.89 ± 0.84	0.78 ± 0.51	1.22 ± 0.69
Germination Rate Index		1.21 ± 0.92	0.23 ± 0.26	0.54 ± 0.58
Vigor Index		1.37 ± 0.69	0.32 ± 0.34	1.62 ± 0.73

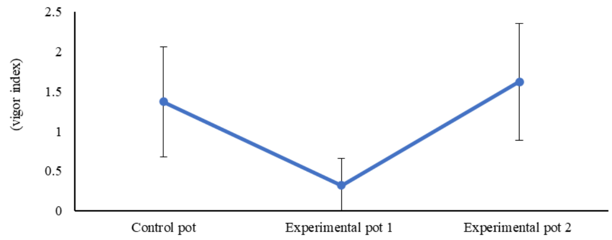

Fig 5: Vigor index from control and experimental pots

Fig 6: Photograph showing stages of allelopathic effects on P. sativum by *T. diversifolia* during the experiment. (a) First day of germination (b) P. sativum after the 60th day (c) Three replicates of P. sativum from each pot were harvested for measurement.

Biomass

Biomass production and distribution an important mechanisms to which plant species can survive under environmental stress (Musyimi *et al.,* 2015). A greater leaf area could result in a larger surface area for light harvesting, which would enhance photosynthesis, biomass gain, and shoot growth (Bano *et al.,* 2012). According to the findings, the experimental pot 2 seedling biomass (1.39 ± 0.17) is greater than that of the control (0.71 ± 0.09) and experimental pot 1 (0.66 ± 0.21) this may be due to greater leaf surface area which increases the biomass production as compared to the other pots (Fig. 4). Similarly, Oluwafemi and Olumide (2013) reported that control pot has the lowest biomass as compared to the other pots. Whereas, Otusanya *et al., (*2015) reported that the biomass seedling is highest in the control regime on the germination of *Amaranthus cruentus*.

Germination index, germination rate index and vigor index

The germination index and germination rate index are the measurements of the percentage and speed of germination, which could differ due to the response of temperature and light regime (Kader, 2005; Javaid, 2018). Table 2 showed that both the germination index and germination rate index of *P. sativum* was highest in the control pot than the experimental pots. Therefore, the higher the value of the germination rate index the greater the rate of germination.

The seed vigor index describes a seed's capacity to emerge quickly, uniformly, and produce high-quality vigorous seedlings (Mangena, 2021). It is associated with many physiological characteristics, including root and shoot length, dry weight of the seedling, germination rate, radicle length, root activity, coleoptile length, mesocotyl length, germination potential, germination index, and time for 50% germination (Zhao *et al.,* 2019; Barik *et al.,* 2022). The study reveals that the experimental pot 2 (1.62 ± 0.73) has the highest vigor index followed by control pot (1.37 ± 0.69) and experimental pot 1(0.32 ± 0.34) (Fig. 5). The diagrammatic representation the pot experiment is shown in Fig.6. In totality, the results indicate that the forest soil has potential of producing high quality seeds.

Conclusion

The present bioassay experiment demonstrated that the growth and germination of *Pisum sativum* were higher in control soil compared to soil infested with *Tithonia diversifolia*. The findings indicate that *T. diversifolia* exerts strong stimulatory allelopathic effects on early growth and developmental parameters of *P. sativum*, particularly during the initial germination phase; however, these effects tend to normalize at later stages. Notably, the infested soil also promoted seedling height and shoot elongation.

Interestingly, *T. diversifolia*-infested soil within moderately disturbed sites exhibited the highest potential for rapid emergence and production of high-quality seeds. These results suggest that such soil may be suitable for agricultural cultivation, although delayed germination may occur under conditions of heavy infestation due to allelopathic interference—an area warranting further investigation. Additionally, leaf leachates from *T. diversifolia* may enhance soil fertility, potentially benefiting crop productivity.

To better understand the allelopathic mechanisms of *T. diversifolia*, future research should focus on its phytochemical composition, particularly in relation to edible crops with implications for nutrition and food security. This knowledge would facilitate sustainable management strategies for *T. diversifolia* across the Indo-Myanmar biodiversity hotspot. Furthermore, expanded bioassay studies involving diverse food crops, along with advanced characterization of secondary metabolites or allelochemical compounds, are recommended to broaden the scope and impact of this research.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualization and motivation (PKR).Writing—Original draft preparation (PKR, Vanlalruati, and RBS). Methodology (PKR, Vanlalruati, and RBS). Writing—Review and editing (PKR & Vanlalruati). Incorporating Revisions/suggestions of reviewers, Supervision, Critical editing, and procuring Project grants (PKR)

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