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## Weedicidal Activity of *Trichoderma harzianum* against Some Weeds of Mash (*Vigna mungo* L.)

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### ABSTRACT

Mash bean (*Vigna mungo* L.) is an important food crop of many areas of the world. It belongs to family Leguminosae and is a dual season crop (cultivated in kharif and spring season both). It is an important source of food for humans and animals. Being a leguminous crop it also fixes the nitrogen of the environment and enhance the fertility of the land. Mash bean face many weeds that compete the crops due to lack of weed management knowledge and results into reduction of crop yield. *Trianthema portulacastrum* (W1) ( family Aizoaceae ), *Amaranthus viridis* (W2)(family Amaranthaceae ), *Euphorbia hirta* (W3) (family Euphorbiaceae ), *Parthenium hysterophorus* (W4)( family Asteraceae), and are some common weeds of mash. These weeds may affect the seed germination as well as the plant growth of mash crop. Chemical herbicides are commonly used to overcome the weeds of crops but they are hazardous for health. Some common herbicides are fenoxaprop-P-ethyl, clodinafop-propargyl + safener cloquintocet, mesosulfuron-methyl, triallate, difenzoquat, diclofop, and imazamethabenz. These causes damage to the main crop as well along with weeds. Biologist are in the search of biological control instead of chemicals. *Trichoderma harzianum* as an alternative weedicidal agent against four major weeds. In the present research work a beneficial fungus *Trichoderma harzianum* along with weeds extracts applied on the mash seeds in the laboratory bioassay. Positive weed treatments (+0.5 to +3) significantly enhanced germination percentages (up to 100%), seedling length (up to 16.25 cm), fresh weight (up to 7 g), and dry weight (up to 1.2 g) compared to negative treatments (-0.5 to -3) and controls. These results indicate that, in the absence of live competition, decomposed weed material can act as a biostimulant, likely by improving nutrient availability and substrate conditions. While *T. harzianum*'s direct weedicidal activity requires further elucidation.

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### INTRODUCTION

The cultivation of mash (*Vigna mungo*), a vital legume crop for protein and soil nitrogen fixation, is persistently constrained by aggressive weed competition (Diatta 2020). Weeds not only usurp essential resources like light, water, and nutrients, but also serve as hosts for pests and pathogens, leading to substantial yield and

economic losses (Sultana et al., 2024). Among the most prevalent and problematic weeds in mash agro-ecosystems are Carpet weed (*Trianthema portulacastrum*), Slender amaranth (*Amaranthus viridis*), Red spurge (*Euphorbia hirta*), and Carrot weed (*Parthenium hysterophorus*). *Trianthema portulacastrum* is a prolific, succulent broadleaf weed

that forms dense mats, while *Amaranthus viridis* is a fast-growing, high seed-producing competitor. *Euphorbia hirta*, though sometimes having medicinal value, aggressively colonizes fields, and *Parthenium hysterophorus* is an infamous invasive species known for its allelopathic effects, human health hazards, and severe crop suppression (Kushal et al., 2024).

Conventional weed management predominantly relies on synthetic herbicides, which pose escalating concerns regarding environmental toxicity, soil and water contamination, evolution of herbicide-resistant weed biotypes, and human health risks. This necessitates a paradigm shift towards sustainable and ecologically benign Integrated Weed Management (IWM) strategies. In this context, biological control using fungal antagonists presents a promising alternative (Parven et al., 2025).

The soil-borne fungus *Trichoderma harzianum* is a well-established biocontrol agent against numerous phytopathogens, celebrated for its rhizocompetence, plant growth-promoting qualities, and ability to induce systemic resistance in plants (Taheri et al., 2025). Its herbicidal potential, however, remains an underexplored frontier. The mechanism of weed suppression by *Trichoderma* spp. may involve a combination of mycoparasitism, the production of cell wall-degrading enzymes (chitinases, glucanases) and phytotoxic metabolites, and competitive exclusion. Evaluating the weedcidal activity of *T. harzianum* against these four economically significant weeds could unveil a novel, sustainable tool for mash cultivation. This study aims to investigate the efficacy of *Trichoderma harzianum* in suppressing the germination and early seedling growth of *Trianthema portulacastrum*, *Amaranthus viridis*, *Euphorbia hirta*, and *Parthenium hysterophorus*, thereby contributing to the development of an eco-friendly component for weed management in legume-based cropping systems.

## MATERIALS AND METHODS

The experiment started with the collection and preparation of the target weed species: Carpet weed (*Trianthema portulacastrum*) W1, Slender amaranth (*Amaranthus viridis*) W2, Red spurge (*Euphorbia hirta*) W3, and Carrot weed (*Parthenium hysterophorus*). W4 Mature plants of each species were gathered from mash fields, with seeds carefully harvested, cleaned, and dried, while aerial parts (leaves and stems) were separately

collected, washed, and air-dried in shade for the preparation of aqueous extracts. For the production of plant extracts, the dried plant material of each weed was ground into a fine powder using a mechanical grinder. A measured quantity of this powder was subjected to maceration in sterile distilled water, followed by filtration through muslin cloth and Whatman No. 1 filter paper to obtain a clear stock extract, which was stored under refrigeration until use.

Concurrently, a pure culture of *Trichoderma harzianum* was multiplied on Malt Extract agar. To support fungal growth for the experimental solutions, a malt extract medium was prepared by dissolving malt extract powder in distilled water, which was then sterilized by autoclaving. Upon cooling, this medium was inoculated with agar plugs from actively growing *T. harzianum* cultures and incubated on a rotary shaker to produce a dense, homogeneous biomass. Seed treatment constituted a critical phase, where healthy, uniform seeds of each weed species were surface-sterilized with a sodium hypochlorite solution. These seeds were then divided into treatment groups. One set was coated with a freshly prepared spore suspension of *T. harzianum*, while control groups were treated with an equal volume of sterile distilled water. For the bioassay, ten seeds of *Vigna mungo* were placed in each sterile Petri dish lined with Whatman filter paper. The filter paper in each dish was uniformly moistened with 3 ml of the respective experimental solution. All plates, including controls, were then arranged in a completely randomized design and maintained at ambient room temperature ( $25 \pm 2$  °C) for an experimental duration of 15 days, with periodic monitoring. Finally, data collection was systematically carried out. Germination counts were recorded daily. Upon germination completion at the end of the incubation period, seedling length (root and shoot) was measured using a standard scale. Seedling fresh biomass was immediately weighed after careful blotting, and for dry biomass, the seedlings were placed in a hot air oven at a constant temperature (70°C for 48 hours) until a stable weight was achieved, providing a measure of plant growth.

## RESULTS

The quantitative dataset revealed distinct and contrasting effects between the experimental treatment groups. The data clearly delineate the impact of weed incorporation alone from the combined influence of

weeds and *Trichoderma harzianum* on the germination and early growth of *Vigna mungo*.

Treatments designated with negative values (-0.5 to -3) represented the incorporation of weed biomass at increasing concentrations in the absence of *Trichoderma harzianum*. In these weed-only conditions, a baseline level of growth promotion was observed. Germination percentages (GERW1–GERW4) ranged from 65% to 90%, indicating that the decomposing weed material itself provided some stimulatory effect, likely through nutrient release and improved substrate conditions. Corresponding growth parameters seedling length (SLW1–SLW4), fresh weight (SFW1–SFW4), and dry weight (SDW1–SDW4) showed measurable but moderate values, with maximum seedling elongation reaching approximately 10.5 cm and fresh biomass plateauing below 4 g.

In stark contrast, treatments with positive values (+0.5 to +3), which combined the same gradient of weed biomass with *Trichoderma harzianum* application, demonstrated a significantly amplified stimulatory effect. Germination under these synergistic treatments consistently reached 95-100% across all concentration levels. The enhancement in vegetative growth was even more pronounced. Seedling length, particularly for SLW3 and SLW4, showed maximum elongation up to 16.25 cm under the +2.5 and +3 combined treatments. Similarly, seedling fresh weight peaked at 7 g, and dry weight accumulation increased to 1.2 g, substantially exceeding

the values recorded in the weed-only (-ve) series. The chord diagram visually reinforces this, showing thicker and more numerous connections emanating from the positive treatment segments to all growth parameters, symbolizing stronger interaction strengths.

The control treatments provided crucial context. The \*\*negative control (-C)\*\*, moistened with water only, showed the lowest values across all measured parameters, establishing the baseline growth without any organic or biological amendments. The \*\*positive control\*\*, which involved the application of *Trichoderma harzianum* without any incorporated weed biomass, displayed a clear growth-promoting effect relative to the water control, confirming the bio-stimulatory properties of the fungus alone. However, its effect was consistently surpassed by every combined treatment (+0.5 to +3), underscoring a synergistic relationship where the presence of decomposing weed biomass enhanced the efficacy of *T. harzianum*, leading to optimal plant performance.

In summary, the results demonstrate a clear hierarchy of effect: \*\*Weed + *Trichoderma* (+ve treatments) > *Trichoderma* alone (Positive Control) > Weed alone (-ve treatments) > Water only (Negative Control)\*\* This indicates that while weed residue alone can function as a soil amendment, its integration with the biocontrol agent *Trichoderma harzianum* yields the most significant positive impact on mash bean establishment and early seedling vigor as shown in Figure 1.

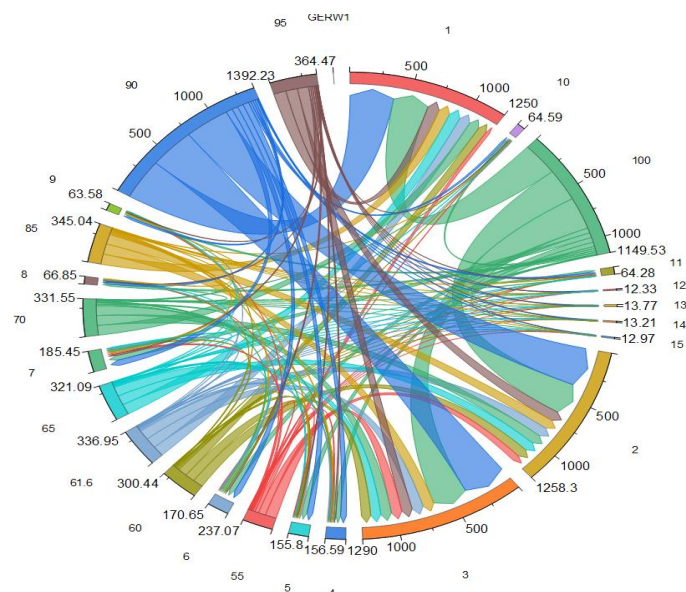


Figure 1. Width of each chord is proportional to the magnitude of the measured value for that parameter-treatment interaction. Red colour positive *Trichoderma harzianum* incorporation treatment (+0.5-+3, blue colour with weeds

treatment (-0.5—3). Thicker chords originating from red/orange segments indicate a stronger stimulatory effect of weed incorporation on *Vigna mungo* growth parameters.

## DISCUSSION

The results of this study present a compelling narrative that reframes the traditional crop-weed-antagonist relationship, revealing a significant synergistic interaction between incorporated weed biomass and the beneficial fungus *Trichoderma harzianum* on the early growth of *Vigna mungo*. The clear performance hierarchy where combined treatments outperformed individual applications, which in turn surpassed controls challenges simplistic pest management paradigms and highlights the complex interplay within agroecosystems. The observed baseline growth promotion in negative treatments (-0.5 to -3; weed biomass alone) aligns with emerging principles in soil ecology, which recognize plant litter as a dynamic nutrient reservoir. The decomposing weed material likely acted as a green manure, gradually mineralizing to release essential nutrients such as nitrogen, phosphorus, and potassium, thereby improving the physicochemical properties of the germination medium (Watthier et al., 2020). This finding is significant as it demonstrates that even problematic plant species can contribute to soil fertility post-mortem, a concept central to organic and conservation agriculture (Han et al., 2024). However, the moderate level of growth promotion indicates that nutrient release from weed residue alone may be slow or sub-optimal for maximizing crop seedling vigor (Ambili et al., 2024).

The pronounced enhancement in all growth parameters under the combined positive treatments (+0.5 to +3) points to a powerful synergy between the organic substrate (weed biomass) and the biological agent (*T. harzianum*). *Trichoderma* spp. are well-documented for their rhizocompetence and multifaceted plant growth-promoting abilities, including phosphate solubilization, production of phytohormones like auxins and gibberellins, and induction of systemic resistance (Sultana et al., 2024). We posit that the incorporated weed biomass served as a preferred carbon source, fueling the metabolic activity and proliferation of *T. harzianum* in the spermosphere (the zone surrounding the seed). This enriched microbial activity likely accelerated the decomposition and nutrient cycling of the weed residue, making minerals more bioavailable to the germinating mash bean seedling in a readily assimilable form (Mahey et al 2024). Furthermore, the

physical improvement of the substrate structure by the fungus, through hyphal networking, would enhance root exploration and water retention, jointly explaining the superior seedling length and biomass accumulation.

The performance of the positive control (*Trichoderma* alone) confirms the fungus's inherent bio-stimulatory potential, as established in numerous studies (Singh et al., 2024; Sharma et al., 2025). However, its inferior results compared to any combined treatment underscore a critical limitation: the efficacy of microbial inoculants is often constrained by the availability of organic carbon in the soil. The provided weed biomass effectively removed this constraint, creating a nutrient-rich niche that allowed the introduced *Trichoderma* population to thrive and exert its full beneficial potential (Sultana et al., 2024). This has direct implications for the practical application of biocontrol agents, suggesting that their success may be greatly amplified when integrated with organic amendments.

From an integrated weed management (IWM) perspective, these findings illuminate a strategic pathway for transforming a liability into an asset. Instead of viewing weed eradication as the sole objective, our results support a management strategy that involves the timely mechanical control of weeds before seed set, followed by their *in-situ* incorporation as mulch amended with *T. harzianum*. This approach achieves multiple objectives simultaneously. It suppresses live weed competition, adds organic matter to the soil, and leverages the residue to boost the activity of a beneficial microbe that further promotes crop growth (Asif et al., 2024). This aligns with the principles of circular agriculture and sustainable intensification, where waste is minimized, and ecological processes are harnessed.

In conclusion, this study demonstrates that the interaction between *Trichoderma harzianum* and incorporated weed biomass is synergistic rather than antagonistic, leading to significantly improved mash bean establishment. It advocates for a paradigm shift in weed management—from unilateral suppression to integrated resource management. Future research should validate these promising *in-vitro* results under field conditions, investigate the specificity of interactions

with different weed species, and develop practical protocols for the co-application of fungal biocontrol agents with weed-derived organic amendments in legume production systems.

### CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

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