



EFFICACY OF TALC-BASED TRICHODERMA FORMULATIONS FOR THE MANAGEMENT OF SOYBEAN WILT AND ENHANCEMENT OF PLANT GROWTH UNDER POT CULTURE CONDITIONS

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ABSTRACT:

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *glycines*, is one of threat to soybean (*Glycine max*). This study investigates the biocontrol potential of indigenous *Trichoderma* spp. formulated with different carrier materials, focusing on talc-based formulations for their efficacy under pot culture conditions. From thirty rhizospheric soil samples, twenty-five *Trichoderma* isolates were obtained, and three superior antagonistic strains (TR2, TR3, and TR23) were selected based on dual culture assays. These isolates were mass-multiplied using six carrier substrates (talc, lignite, charcoal, sawdust, compost, and fly ash), and their shelf life was evaluated over 180 days. Talc emerged as the most effective carrier, maintaining the highest colony-forming units (CFU/g) across all time intervals. The results suggest that talc-based formulations of native *Trichoderma* isolates, particularly TR2, offer a sustainable and eco-friendly alternative to chemical fungicides.

KEYWORDS:- Trichoderma, Soybean, Talc-based formulation, Biocontrol, Shelf life.

INTRODUCTION:

Soybean (*Glycine max* (L.) Merr.) is the most widely cultivated legume globally, valued for its protein-rich seeds used in food, feed, and industry (Carroll et al., 1985). Domesticated in Eastern Asia around 6000 years ago, it has been extensively improved through plant breeding for yield, adaptability, and disease resistance. Its wide maturity range enables cultivation across diverse climates. Advances in genomics and biotechnology have established it as a model legume species (Schmutz et al., 2010). However, diseases like Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *glycines*, one of aspect that reduce yield. Thus, sustainable, eco-friendly

alternatives to chemical control are urgently required.

Biological control using antagonistic fungi such as *Trichoderma* spp. has gained prominence as a sustainable alternative to chemical fungicides. These fungi suppress a wide range of plant pathogens through mechanisms including competition for nutrients and space, mycoparasitism, secretion of hydrolytic enzymes, and production of antifungal metabolites (Vinale et al., 2008). In addition, *Trichoderma* spp. are well-documented for their plant growth-promoting effects and induction of systemic resistance, thereby enhancing overall plant health (Shoresh et al., 2010). Among these, talc is widely favored for its inert nature, ease of

handling, and ability to preserve high spore viability over extended periods.

This study aimed to mass multiply and formulate three potent *Trichoderma* isolates using six carrier materials, assess their shelf life and viability.

MATERIALS AND METHODS

Collection and Processing of Soil Samples

Rhizospheric soil samples were collected from 30 soybean-growing sites across the Vidarbha region of Maharashtra, including districts such as Akola, Amravati, Yavatmal, Wardha, Washim, Buldhana, Nagpur, Chandrapur, Gadchiroli, and Bhandara. At each location, three composite soil samples were collected in triplicate from the rhizosphere (10–20 cm depth) of healthy soybean plants, avoiding surface litter. The fields were visually divided based on crop growth, soil texture, and topography to ensure homogeneity during sampling. Collected soil samples were cleaned for debris, bulked and reduced to 100–200 g using the quartering method. The processed samples were placed in labelled paper bags and transported to the laboratory for fungal isolation.

Isolation, Purification, and Maintenance of *Trichoderma* spp.

Isolation of *Trichoderma* was performed using the serial dilution plate technique (Johnson, 1957). Soil suspensions were prepared in sterile distilled water and serially diluted up to 10^{-7} . Aliquots (1 ml) from the final dilution were plated on *Trichoderma* Specific Selective Medium (TSSM), which contained glucose (3.0 g), K_2HPO_4 (0.9 g), NH_4NO_3 (1.0 g), $MgSO_4$ (0.2 g), KCl (0.5 g), Rose Bengal (0.033 g), Metalaxyl (0.2 g), PCNB (0.2 g), chloramphenicol (0.25 g), and agar (15 g) per liter. Medium was autoclaved at 15 psi for 20–25 min and poured into Petri plates under aseptic conditions. Following incubation at $28 \pm 1^\circ C$ for one week, emerging colonies of *Trichoderma* were subcultured on Potato Dextrose Agar (PDA) for

purification. Pure isolates were obtained through single-spore isolation (Tuite, 1969) and maintained on PDA slants at $28 \pm 1^\circ C$ for further study. A total of 25 isolates were purified and preserved for characterization and antagonistic assays.

Isolation and Identification of *Fusarium oxysporum* f. sp. *glycines*

Wilt-infected soybean plants showing typical symptoms (yellowing, leaf drooping, vascular discoloration, and whole plant wilting) were collected from multiple fields in the Nagpur region during the kharif season. Infected root segments (1–2 mm) were excised, surface-sterilized with 1% sodium hypochlorite for one minute, rinsed thrice with sterile distilled water, blotted dry, and transferred to PDA plates. Fungal growth emerging from tissues was subcultured, and pure cultures were obtained via single hyphal-tip isolation. Cultures were incubated at $25 \pm 2^\circ C$ and maintained on PDA for further study. Identification of *Fusarium oxysporum* f. sp. *glycines* was based on morphological characteristics such as white to violet floccose colonies and microscopic traits. Taxonomic confirmation was made using descriptions by Leslie & Summerell (2006).

Mass Multiplication and Formulation of *Trichoderma* Using Various Carrier Materials

To evaluate the effectiveness of different carrier materials in supporting the survival and activity of *Trichoderma*, mass multiplication of the three most potent antagonistic isolates (isolate TR2, isolate TR3 and TR 23) was carried out using six carrier substrates: talc powder, lignite, charcoal, sawdust, compost and fly ash. These carriers were selected based on their physical properties, organic matter content, and potential to enhance the shelf life and efficacy of *Trichoderma*-based formulations. All carrier materials were first dried, powdered (where applicable), and sterilized by autoclaving at 15 psi for 30 minutes on two

consecutive days to eliminate microbial contamination. Separately, the *Trichoderma* isolates were cultured in Potato Dextrose Broth (PDB) and incubated for 10–12 days at $28 \pm 2^\circ\text{C}$ to achieve optimal spore production. The spore suspensions were filtered through sterile muslin cloth to remove mycelial mats and adjusted to a concentration of approximately 1×10^7 conidia/ml. Each sterilized carrier was then mixed thoroughly with the *Trichoderma* spore suspension at a moisture content of 25–30% to ensure uniform colonization. The inoculated mixtures were incubated under ambient laboratory conditions for 10–15 days, with periodic manual mixing to maintain aeration. The resulting bioformulations were packed in sterile polythene bags, sealed, and stored at room temperature for subsequent shelf-life studies, viability assessment (cfu/g), and efficacy testing against *Fusarium oxysporum* f. sp. *glycine* under in vivo conditions.

Shelf Life Evaluation of *Trichoderma* Formulations

To assess the shelf life of *Trichoderma* formulations prepared using different carrier materials (talc, lignite, charcoal, sawdust, compost, and fly ash), a systematic protocol was followed for determining the viable propagule count (cfu/g) at regular intervals of 0, 30, 60, 90, 120, 150, and 180 days' post formulation.

PROCEDURE:

1. Storage Conditions: Formulated *Trichoderma* products were stored in sterile low-density polythene bags, sealed, and kept at room temperature ($25 \pm 2^\circ\text{C}$) under dry conditions away from direct sunlight.
2. Sampling Intervals: Viability of *Trichoderma* was assessed at 0, 30, 60, 90, 120, 150, and 180 days after preparation.

3. Serial Dilution and Plating: At each interval, 10 g of the formulation was taken from each carrier type. The sample was suspended in 90 ml of sterile distilled water in a 250 ml conical flask and shaken thoroughly on a rotary shaker for 30 minutes. Serial dilutions up to 10^{-5} or 10^{-6} were prepared. 1 ml of each dilution was plated on *Trichoderma* Selective Medium (TSM) using the pour plate technique. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 5–7 days.
4. Colony Counting: After incubation, colonies with typical *Trichoderma* morphology were counted. The number of viable spores was expressed as colony forming units per gram (cfu/g) of the carrier material.
5. Data Recording: Data were recorded and tabulated for each carrier at every time point. A minimum cfu/g of 1×10^6 is generally considered acceptable for commercial bioformulations.

RESULTS AND DISCUSSION

Collection and Isolation of *Trichoderma* spp. from Rhizospheric Soils

A total of 30 rhizospheric soil samples were systematically collected from the root zones of diverse crops cultivated under different agro-climatic zones across the Vidarbha region of Maharashtra. The selected sites encompassed a wide range of soil textures, organic matter levels, cropping histories, and field conditions, thereby enabling a comprehensive representation of native soil microbial diversity. Each soil sample was collected aseptically using sterilized tools, labeled from S1 to S30, and transported to the laboratory under chilled and sterile conditions to prevent microbial contamination and maintain sample integrity. The isolation of *Trichoderma* spp. was performed using the serial dilution

method, followed by inoculation onto Trichoderma Specific Medium (TSM), which selectively supports the growth of Trichoderma while suppressing other microbial contaminants. After 5–7 days of incubation at $28 \pm 1^\circ\text{C}$, colonies showing typical Trichoderma like morphology characterized by rapid growth, green pigmentation, and concentric rings were observed in 25 out of 30 samples. The remaining five samples showed no visible fungal growth, either due to an absence of viable propagules or possible suppression by inhibitory factors present in the soil. The absence of fungal colonies

in the five soil samples (S7, S12, S16, S22, and S28) may be attributed to several environmental, biological, or technical reasons. Soil samples with extremely low organic carbon, poor nutrient availability, or residual pesticide and fungicide applications might lack the microbial diversity required to support fungal colonization. The use of general media like PDA may also fail to support the growth of more fastidious fungal strains, highlighting the need for specialized culture conditions when targeting specific taxa such as Trichoderma. (Table 1)

Sample code	Location	Trichoderma recovered	Isolate code	Growth of colony observed
S1	Wardha	YES	TR1	Olive Green
S2	Nagpur	YES	TR2	Green
S3	Bhandara	YES	TR3	Dark Green
S4	Amravati	NO	-	No growth observed
S5	Gondia	YES	TR4	Pale Green
S6	Amravati	NO	-	No growth observed
S7	Chandrapur	YES	TR5	Green
S8	Akola	YES	TR6	Dark Green
S9	Yavatmal	NO	-	No growth observed
S10	Nagpur	YES	TR7	Pale Green
S11	Nagpur	YES	TR8	Dark Green
S12	Gadchiroli	YES	TR9	Olive Green
S13	Nagpur	YES	TR10	Olive Green
S14	Washim	NO	-	No growth observed
S15	Washim	YES	TR11	White
S16	Washim	YES	TR12	Dark Green
S17	Buldhana	NO	-	No growth observed
S18	Nagpur	YES	TR13	Green
S19	Yavatmal	YES	TR14	Green
S20	Chandrapur	YES	TR15	Pale Green
S21	Nagpur	YES	TR16	White
S22	Wardha	YES	TR17	Green
S23	Akola	YES	TR18	Yellow
S24	Akola	YES	TR19	Yellow
S25	Yavatmal	YES	TR20	Green
S26	Yavatmal	YES	TR21	Yellow
S27	Akola	YES	TR22	Pale Green
S28	Amravati	YES	TR23	Dark Green
S29	Washim,	YES	TR24	Pale Green
S30	Wardha	YES	TR25	Dark Green

Table 1: Isolation and Morphological Confirmation of *Trichoderma* spp. from Rhizosphere Soil Samples Collected from Vidarbha Region

Collection, Isolation, and Identification of *Fusarium oxysporum* f. sp. *glycines*

To obtain the pathogen for antagonism studies, diseased soybean plants showing typical symptoms of *Fusarium* wilt were collected. The collected samples were labeled, documented, and brought to the laboratory under sterile, cool conditions for pathogen isolation. Small sections of discolored root tissues were surface-sterilized with 1% sodium hypochlorite, rinsed with sterile distilled water, and aseptically plated onto PDA medium. After 48–72 hours of incubation at $25 \pm 2^\circ\text{C}$, white to pale pink fungal growth emerged, which later developed violet pigmentation on the reverse a characteristic feature of *Fusarium* spp. Pure cultures were obtained using the hyphal tip method and maintained on PDA slants. Microscopic examination confirmed the identity of the fungus as *Fusarium oxysporum* f. sp. *glycines*.

Mass Multiplication and Formulation of *Trichoderma* Using Various Carrier Materials

Mass multiplication were carried out using selective isolates of *Trichoderma* using different carrier materials. Out of the twenty *Trichoderma* isolates evaluated through dual culture assay against *Fusarium oxysporum* f. sp. *glycine*, three isolates (TR2, TR3 and TR23) exhibiting the

highest antagonistic efficacy were selected for mass multiplication studies. These isolates demonstrated superior inhibition of pathogen growth, ranging from 70% to 77%, along with rapid mycelial growth, profuse sporulation, and consistent morphological characteristics. Additionally, these isolates were identified up to species level through 18S rRNA gene sequencing, confirming their identity as potent biocontrol species. The selected three isolates were subsequently utilized for development of carrier-based formulations using different carrier materials to evaluate their shelf-life, viability, and efficacy under storage and application conditions.

Shelf Life Evaluation of *Trichoderma* Formulations

The shelf-life study was conducted using six different carrier materials under ambient storage conditions. The three most promising antagonistic isolates TR2, TR3, and TR23 were mass-multiplied and formulated separately with talc, lignite, charcoal, sawdust, compost, and fly ash. Periodic observations were recorded up to 180 days to evaluate colony-forming unit (CFU) counts, providing insights into the stability and efficiency of each formulation.

Table 2. Shelf life of *Trichoderma* isolate TR2 in different carrier-based formulations

Sr. No.	Substrate	Days of storage at room temperature (CFU 10^6 /g of the substrate)						
		0 Days	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
1.	Talc	170.25a	155.25a	142.00a	126.50a	93.25a	73.00a	47.00a
2.	Lignite	167.00e	133.75d	110.25c	91.00d	66.75d	52.25c	34.00c
3.	Charcoal	169.00b	134.50c	109.00d	90.00d	65.00e	47.75f	22.50e
4.	Sawdust	166.50f	105.75j	91.25i	72.00g	53.25g	40.75g	20.00ef
5.	Compost	165.00g	111.00h	89.75j	69.75h	52.75g	41.50g	19.25fg

Sr. No.	Substrate	Days of storage at room temperature (CFU 10^6 /g of the substrate)						
		0 Days	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
6.	Fly ash	167.75c	123.25f	104.00f	85.00e	61.50f	39.25h	17.00g

Data illustrated in table 2 showed that the shelf life performance of *Trichoderma* isolate TR2 formulated with six different carrier materials and stored at room temperature over a 180-day period. Among all tested substrates, the talc-based formulation consistently supported the highest population of viable propagules, starting from 169.50×10^6 CFU/g at day 0 and maintaining 45.50×10^6 CFU/g at 180 days. Lignite and charcoal also demonstrated good compatibility, retaining 31.75 and 19.50×10^6 CFU/g at the end of the storage period, respectively. In contrast, formulations prepared

with sawdust, compost, and fly ash showed comparatively lower viability, with final CFU counts of 18.75, 18.25, and 16.25×10^6 respectively. The data clearly indicate that talc is the most effective carrier for maintaining shelf stability of isolate TR2, followed by lignite. These results highlight the influence of substrate composition, porosity, and moisture-holding capacity on the survival of *Trichoderma* over time. The superior performance of talc may be attributed to its inert nature, favorable surface properties, and ability to maintain optimal moisture conditions for spore viability.

Table 3. Shelf life of *Trichoderma* isolate TR3 in different carrier-based formulations

Sr. No.	Substrate	Days of storage at room temperature (CFU 10^6 /g of the substrate)						
		0 Days	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
1.	Talc	158.50a	142.75a	127.25a	111.50a	78.00a	60.50a	35.75a
2.	Lignite	153.25d	119.00d	97.00c	78.25d	54.25d	41.50c	22.75c
3.	Charcoal	155.00c	121.50c	95.00d	76.25d	52.50e	37.75f	17.75e
4.	Sawdust	152.00f	92.25j	75.50i	59.75g	41.25g	29.25g	14.75ef
5.	Compost	150.75g	98.25h	73.00j	56.50h	40.25g	30.00g	13.25fg
6.	Fly ash	154.00e	110.00f	89.00f	70.00e	47.00f	27.50h	11.25g

The data presented in table 3 on the shelf life of *Trichoderma* isolate TR2 formulated with various carrier materials and stored at room temperature over a period of 180 days. Among all the carriers tested, talc-based formulation recorded the highest initial population of 158.50×10^6 CFU/g at day 0, which gradually declined to 33.50×10^6 CFU/g by the end of the storage period. Though the values were consistently lower than those observed for isolate TR3, talc still proved to be the most suitable carrier for maintaining viable propagules of isolate TR2. Lignite and charcoal followed in efficacy, showing CFU counts of 25.75

and 17.25×10^6 at 180 days, respectively. The remaining carriers—sawdust, compost, and fly ash—exhibited sharper declines in viability, with fly ash supporting the least survival (12.00×10^6 CFU/g at 180 days). The overall trend indicates that isolate TR3 demonstrated a relatively reduced shelf life across all carrier materials compared to isolate TR2, possibly due to inherent differences in physiological traits or sporulation potential. Nonetheless, the data affirm the utility of talc and lignite as preferred substrates for developing shelf-stable bioformulations of this isolate.

Table 4. Shelf life of *Trichoderma* isolate TR23 in different carrier-based formulations

Sr. No.	Substrate	Days of storage at room temperature (CFU 10^6 /g of the substrate)						
		0 Days	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
1.	Talc	163.25a	147.00a	132.00a	117.75a	85.25a	66.25a	39.00a
2.	Lignite	160.50d	125.50d	103.75c	83.50d	59.00d	45.25c	26.50c
3.	Charcoal	161.75c	128.25c	100.25d	81.75d	58.00d	41.75d	21.00d
4.	Sawdust	158.00e	97.50i	81.25h	63.50g	45.50f	33.25f	16.75e
5.	Compost	156.75f	102.00h	79.50i	60.25h	43.00f	33.75f	15.50ef
6.	Fly ash	159.00e	114.75f	95.25f	74.25e	50.00e	35.50e	13.75f

Table 4 presents the shelf-life data of *Trichoderma* isolate TR23 formulated with various carrier materials over a storage period of 180 days at ambient conditions. Similar to isolate TR2, the talc-based formulation showed the highest initial and sustained viability, with 168.25×10^6 CFU/g at day 0 and 40.75×10^6 CFU/g at 180 days, indicating its potential as a superior carrier for prolonged shelf life. Lignite and charcoal supported moderate survival, maintaining 30.25 and 21.50×10^6 CFU/g,

respectively, by the end of the storage period. The CFU count for fly ash-based formulation (18.25×10^6 CFU/g) was slightly higher than sawdust (17.75×10^6 CFU/g) and compost (16.25×10^6 CFU/g), indicating better support for isolate TR23 in fly ash compared to the other organic substrates. The population trends observed across time intervals show that isolate TR23 exhibited intermediate stability compared to isolates TR2 and TR3, with modest fluctuations across different carriers, reflecting its strain-

specific resilience and adaptability. This outcome suggests that while talc remains the best performing carrier, the isolate's overall survival also depends on its inherent physiological characteristics and compatibility with carrier material properties.

CONCLUSION

The shelf life of *Trichoderma* isolates TR1, TR2, and TR3 was evaluated using six carrier materials over 180 days. Talc-based formulations exhibited the highest CFU counts, maintaining viable populations across all isolates. TR1 showed the best viability (45.50×10^6 CFU/g), followed by TR3 (43.50×10^6 CFU/g) and TR2 (29.75×10^6 CFU/g), indicating talc's superior performance due to its inertness, moisture retention, and protection from stress. Lignite and charcoal showed moderate viability for TR1 and TR3, attributed to their porous structure and organic content. Compost, sawdust, and fly ash were less supportive, particularly for TR2, with viability dropping below 20×10^6 CFU/g at 180 days.

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