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QUALITY CONTROL OF BIO-PESTICIDES

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Abstract: Biopesticides, encompassing microbial, botanical, and biochemical products, are increasingly recognized as essential components of sustainable pest management. Despite their ecological advantages, inconsistent field performance and variable product quality remain significant challenges, often attributed to inadequate quality control during production and formulation. This study critically examines the methodologies employed in the quality control of biopesticides, including microbial viability assays, contaminant detection, potency measurement, and formulation stability analysis. Emphasis is placed on the role of Good Manufacturing Practices (GMP) and compliance with national and international regulatory standards. The paper also highlights emerging techniques, such as molecular tools and omics technologies, that enhance the accuracy and efficiency of quality control processes. Addressing the current limitations and proposing standardized protocols are crucial for improving the reliability, market acceptance, and wider adoption of biopesticides in integrated pest management systems.

Keywords: Biopesticides, Quality Control, Microbial Viability, GMP Standards, Contaminant Detection, Sustainable Agriculture, Integrated Pest Management.

Introduction

PEST

Pests are often animals or insects that prey on crops and ruin their production. The standard method for eliminating them involves the use of many insecticides or herbicides. Nonetheless, pesticide usage exacerbates the situation and creates even more immense complications.

Integrated pest management

Integrated Pest Management (IPM) is an ecosystem-based strategy that tries to prevent pests or damage they cause in the long term via the use of biological management, changes to habitat, adjustments to cultural practices, and the adoption of resistant cultivars. It is a method for integrating pest management approaches. This approach strictly adheres to established protocols for the use of pesticides, and the whole treatment process is designed to eradicate just the specific species of interest (Wagle, 2018).

Trichoderma harzianum"

Whether it's on soil, decomposing wood, or plant debris, the genus Trichoderma is found all over the world. In a broad range of environments, species of Trichoderma may be found predominating in the soil microflora. Because of their fierce competitiveness and varied metabolic capacities, Trichoderma species may be to blame. However, a particularly nasty strain of Trichoderma harzianum is responsible for a major problem with commercial mushrooms. Typically, Trichoderma strains aren't linked with plant illnesses. (published by Kubicek et al. in 2002).

Quality control

This is very necessary if we want farmers to continue believing in the effectiveness of biocontrol products. Because they are living things, the number of microorganisms in a product determines how long it will last. The lowest amount required to bring about efficient biological control of plant diseases is determined by the population burden of the antagonists (Kumar *et al*., 2014).

MATERIAL AND METHODS

Experimental Site and Duration of Study

The Work was carried out in Bio-pesticide Testing Laboratory, Regional Central Integrated Pest Management Centre, Lucknow under Directorate of Plant Protection Quarantine & Storage, Ministry of Agriculture and Farmers Welfare, Government of India. This independent lab work was conducted during period ranging from 1st January, 2025 to 12th February, 2025.

Sample collection

Table 4: Sample has been collected from 3 different sites.

S.No.	Sample collection site	Sample Code
1.	Biopesticide shop in Lucknow	LKO/01
2.	Biopesticide shop in Barabanki	BAR/01
3.	Biopesticide shop in Sitapur	SIT/01
4.	Biopesticide shop in Raebareilly	RAB/01

Photo 1: Samples

Image of sample 1

Image of sample-2

Image of sample-3

Image of sample-4

Cleaning and sterilization of glassware

All the glassware that was used for this process such as test tube, measuring cylinder, conical flask, petri dishes, pipette were cleaned with detergent, then solution of chronic acid and finally all washed with running tap water, to completely remove traces of detergent or acid.

All the glassware were placed in hot air oven at 180 ℃ for complete sterilization.

Conical flask and test tubes were covered with cotton plug and foil paper and kept for autoclaving at 121 ℃ for sterilization and disinfection.

Photo 2: Sterilized Glassware

Preparation of Media / *Trichoderma* Selective Media (TSM):

Sterilized glassware was used for Preparation of *Trichoderma* Selective Media (TSM). In a conical flask of 1 litre, take 1 Litre distilled water and dissolved 3.0g of Glucose, 1.0 g of Ammonium nitrate, 0.9 g of Dipotassium hydrogen phosphate, 0.2 g of Magnesium sulphate heptahydrate, 0.15 g of Potassium chloride, 0.15 g of Rose Bengal, 0.3 g of Metalaxyl, 0.25 g of Chloramphenical and 15.0 g of agar. Stirred properly and shaken continuously to dissolve agar in the flask, then autoclaving at 121 ℃ for 20 minutes for sterilization and disinfection.

Photo 3: pouring media in plates

Photo 4: Autoclaved media

Table 5: Components of Trichoderma Selective Media (TSM)

S. No.	Components	Quantity
1.	Agar	15.0 g.
2.	Glucose	3.0 g.
3.	Ammonium nitrate	1.0 g
4.	Dipotassium hydrogen phosphate	0.9 g.
5.	Magnesium sulphate heptahydrate	0.2 g
6.	Potassium chloride	0.15 g
7.	Rose Bengal	0.15 g.
8.	Metalaxyl	0.3 g
9.	Chloramphenical	0.25 g
10.	Distilled water	1 litre

Surface Sterilized the laminar air flow chamber with 70% alcohol. After cooling put autoclaved conical flask and test tube under the laminar air flow chamber and turned on the UV light for 45 minutes for sterilization. Then poured media in the petri dish in the front of spirit lamp to avoid contamination. Then turned on UV light for 1 hour, after which the media was ready for inoculation. Then proceeded for serial dilution of sample

Serial Dilution

Take 1 g. of product and mix it in 9 ml of sterilized distilled water in a clean and sterilized test tube to make 10^{-1} dilution (1:10)

↓

Shake well and take 1 ml. of the suspension to 9 ml. of sterile water in a tube to make 10^{-2} dilution (1:100)

↓

Make four more serial dilutions in the same way to get 10^{-6} dilution

↓

Transfer 1 ml of this suspension to sterile Petri Plates and add 15-20 ml of sterilized, melted and cooled Trichoderma spp. selective media

↓

Rotate the plates gently and allow it to solidify. Incubate the Perti plates in BOD incubator under the fluorescent illumination at 25 ℃± 2 ℃ and R.H. at 65% ± 5 % for five to seven days

Photo 5: preparation of serial dilution

Table 6: SPECIFICATION & METHOD OF ANALYSIS OF Trichoderma spp.

Particulars	Specification
Form and appearance	Physical state and colour of samples
pH	6.0-8.0
CFU/g of the product	2 x 10^{-6} / gm or ml min
Moisture content	8%max
CFU count	Trichoderma 2x10^{-6} CFU /ml or gm (stability at 30°C and 65% RH)
contamination	. Pathogenic contaminants – such as GramNegative bacteria salmonella, shigella, vibrio etc; absent . Other contaminants should not exceed 1x10^{4}/ ml or g . Chemical/ botanical pesticides contaminant; absent.

METHOD OF ANALYSIS

CFU counts by serial dilution and examination under regular compound research microscope with bright field optics.

Estimation of Colony Forming Units count:

Taken 1 g. of product and mixed in 9 ml of sterilized distilled water in a clean and sterilized test tube to make 10-1 dilution (1:10). Shaken well and take 1 ml. of the suspension to 9 ml. of sterile water in a tube to make 10^{-2} dilution (1:100). Make four another serial dilutions were made in the same way to get 10^{-6} dilution. Transferred 1 ml. of this suspension to sterile petri plates and add 15-20 ml. of sterilized, melted and cooled *Trichoderma spp.* Selective Media. Rotated the plates gently and allowed to solidify. Incubated the Perti plates in BOD incubator under the fluorescent illumination at 25 °C ± 2 °C and R.H. at 65% ± 5 % for five to seven days. Observed the development of typical *Trichoderma* spp. colony and calculated the number of colony unit per gram of the sample with the following formula.

PRECAUTIONS

The following precaution were observed during the analysis of *Trichoderma* spp. Samples:

1. Chemicals & Glasswares: Analytical Grade (AR) quality chemicals and Schott Duran or Borosil glassware were used.
2. Cleaning and Sterilization: - The glasswares used for testing were ensured to be clean by washing with detergent like Lavoline and Sterilized in Hot air Oven at 180°C for 3 hours.
3. Sterilization: - Media, distilled water and micro tips were autoclaved at (15 psi) pressure,121°C for 20 minutes.

Calculation

Formula used:

CFU/g.= Average number of colonies/dilution factor

Sample - 1

Table 7. Number of colony forming unit/plates in Sample code (LKO/01)

Dilution factor	**No. of colony forming units/plate**				**average**
Y	R1	R2	R3	R4	
10^{-6}	4	8	4	8	6

Formula used:

CFU/g = average no. of colonies (X)/dilution factor (Y)

$= 6 / 10^{-6}$

Sample – 2

Table 8. Number of Colony forming unit/plate in sample code (BAR-01)

Dilution factor	**No. of colony forming units/plate**				**Average**
Y	R1	R2	R3	R4	X
10^{-6}	1	2	0	1	1

Formula used:

CFU/g= average no. of colonies (X)/dilution factor (Y)

$= 1/10^{-6}$

Sample 3

Table 9. number of colony forming unit/plate in sample code (SIT/01)

Dilution factor	**No. of colony forming unit/plate**				**average**
Y	R1	R2	R3	R4	X
10^{-6}	15	18	14	12	16

Formula used:

CFU/g = average no. of colonies (x)/dilution factor (Y)

$= 16/10^{-6}$

Sample 4

Table 10. number of colony forming unit/plate in sample code (RAB/01)

Dilution factor	**No. of colony forming units/plate**				**average**
Y	R1	R2	R3	R4	X
10^{-6}	8	6	7	12	8.25

Formula used:

CFU/g = average no. of colonies (x)/dilution factor (y)

$= 8.25/10^{-6}$

Other contaminants

Sample 1

Table 11. Number of Other Microbial Contaminants/plates

Dilution factor	**No. of Other Microbial Contaminants/plate**				**average**
Y	R1	R2	R3	R4	
10^{-4}	1	2	0	1	1

Sample 2

Table 12. Number of other microbial contaminants/plates

Dilution factor	**No. of other microbial contaminants /plate**				**average**
Y	R1	R2	R3	R4	X
10^{-4}	4	6	1	5	4

Sample 3

Table 13. number of other contaminants/plates

Dilution factor	**No. of other microbial contaminants/plate**				**average**
Y	R1	R2	R3	R4	X
10^{-4}	1	4	3	2	5

Sample – 4

Table 14. number of other contaminants/plates

Dilution factor	**No. of other microbial contaminants/plate**				**average**
Y	R1	R2	R3	R4	X
10^{-4}	2	3	2	1	2

MOISTURE CONTENT:

Sample -1

Table 15. Moisture content to be determined by the using moisture meter.

Moisture percentage % by Automatic Moisture Analyser Machine			**Mean**
R1	R2	R3	X
2.62	3.89	4.06	3.52

Sample-2

Table 16. Moisture content to be determined by the using moisture meter.

Moisture content % by automatic moisture analyser machine			**Mean**
R1	R2	R3	X
9.6	9.4	9.2	9.43

Sample – 3

Table 17. Moisture content to be determined by the using moisture meter.

Moisture content % by automatic moisture analyser machine			**mean**
R1	R2	R3	X
3.12	4.06	2.98	3.38

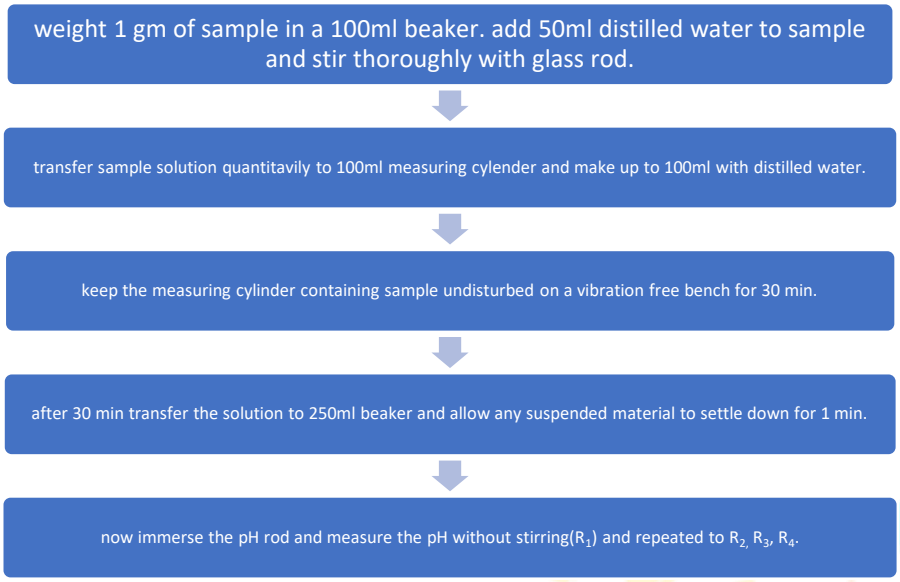
Sample – 4

Table 18. Moisture content to be determined by the using moisture meter.

Moisture content % by automatic moisture analyser machine			**mean**
R1	R2	R3	X
5.71	4.95	4.41	5.02

ESTIMATION OF pH

pH (acidity or alkalinity test) of bio-pesticides is conducted as per



pH meter calculation

average = R_1+R_2+ R_3+R_4/ 4

pH of the suspension is observed by using the meter

Sample- 1

Table 19. pH of the suspension is observed by using the meter.

R1	R2	R3	R4	Average
7.32	7.45	7.71	7.53	7.50

pH of sample = 7.50

sample - 2

Table 20. pH of the suspension is observed by using the meter.

R1	R2	R3	R4	Average
8.33	8.56	8.72	8.41	8.50

pH of sample = 8.50

sample - 3

Table 21. pH of the suspension is observed by using the meter.

R1	R2	R3	R4	Average
7.75	7.53	7.32	7.61	7.55

pH of sample = 7.55

sample - 4

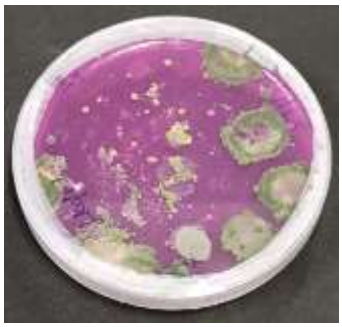
Table 22. pH of the suspension is observed by using the meter.

R1	R2	R3	R4	Average
7.91	7.60	7.81	7.52	7.71

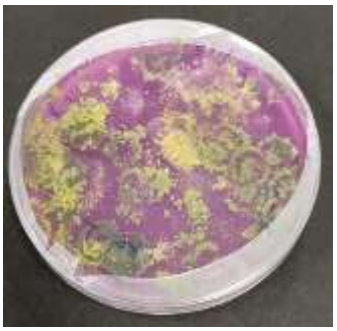
pH of sample = 7.71

Sample-1

10^{-6} plate



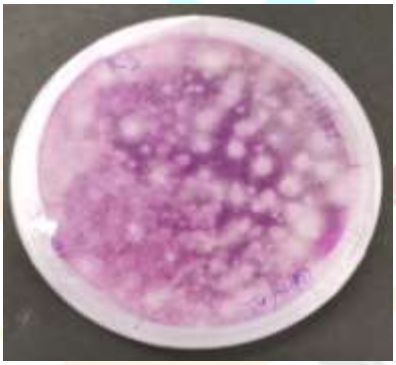
10^{-4} plate



Sample 2

10^{-6} plate

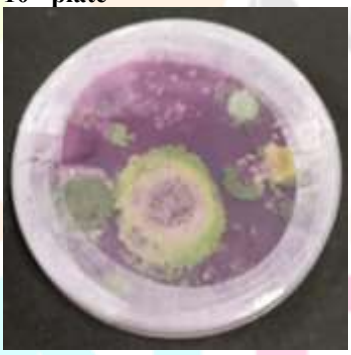
10^{-4} plate



Sample 3

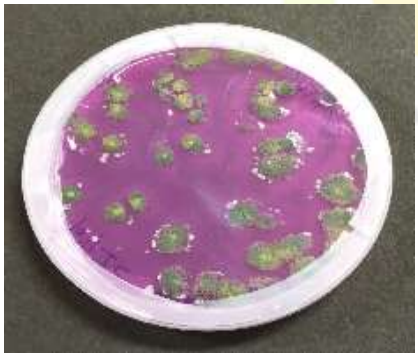
10^{-6} plate

10^{-4} plate



Sample 4

10^{-6} plate



10^{-4} plate

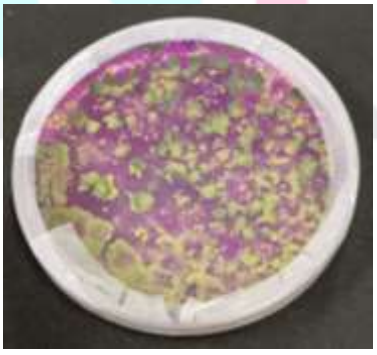


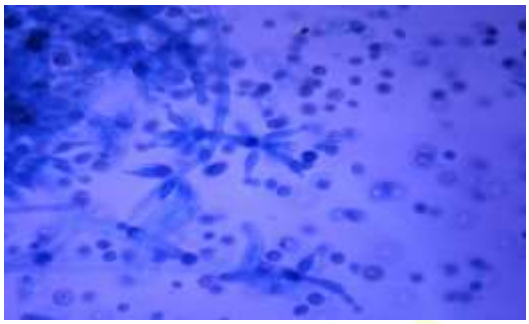
Photo 6: growth of fungus on TSM

Identification:

Slide Preparation:

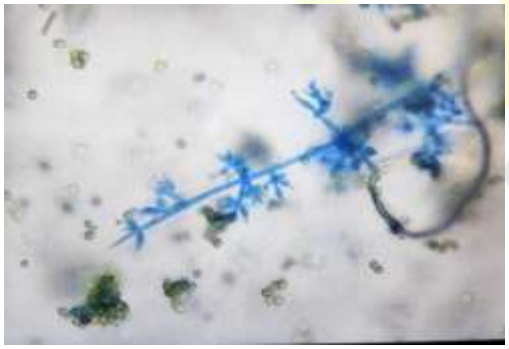
- Put a drop of lactophenol cotton blue stain in the middle of a clean glass slide.
- Using a sterilized loop, needle, or forceps, gently pick up a small piece of the specimen.
- Place the specimen in the drop of stain.
- Gently tease the specimen apart if it is large or dense.
- Lower a coverslip over the specimen, avoiding air bubbles.
- Examine the slide under a microscope.

Photo 7: prepared slide of samples



Trichoderma of sample- 1

Trichoderma of sample- 3



Trichoderma of sample-4

Results

Table 23. Results of samples.

S. No.	Sample Code	CFU/g (x 10^6)	pH	Moisture (%)	Other contaminants/g (x 10^4)
1.	LKO/01	6	7.50	3.55	1
2.	BAR/01	1	8.50	9.43	4
3.	SIT/01	16	7.55	3.38	5
4.	RAB/01	8.25	7.71	5.02	2

Table 23 presents the quality control analysis of four biopesticide samples collected from different locations, designated as LKO/01, BAR/01, SIT/01, and RAB/01. The parameters assessed include microbial load (CFU/g), pH, moisture content, and the presence of other contaminants.

The microbial count, expressed in CFU per gram (2×10^6), varied significantly among the samples. The highest microbial load was observed in sample SIT/01 (16×10^6 CFU/g), indicating a potentially more active biopesticide formulation, whereas the lowest count was found in BAR/01 (1×10^6 CFU/g). The pH values ranged from 7.50 to 8.50, with BAR/01 exhibiting the most alkaline pH, Moisture content, an important factor affecting the shelf life of biopesticides, was highest in BAR/01 (9.43%) and lowest in SIT/01 (3.38%).

Contaminant levels (1×10^4/g) also varied, with SIT/01 having the highest presence of other contaminants (5×10^4/g), potentially indicating compromised formulation or storage conditions. In contrast, LKO/01 recorded the lowest level of contamination (1×10^4/g).

These results highlight the variability in the quality of biopesticide formulations across different samples. Higher microbia1 counts with controlled moisture and low contamination are desirable indicators of an effective and stable biopesticide product

Discussion

Quality control of Bio-pesticides becomes very important in the present scenario as farmers are becoming more and more aware and opting for eco-friendly and chemical free insecticides and fungicides. Poisonous chemical pesticides are damaging the environment as well as human health. Spurious and mis-branded chemical and microbial bio-pesticide pose a great risk to agriculture and its productivity. Thus, maintaining the quality of Bio-pesticides is the foremost important task for government organisations and other stakeholders.

The samples tested from four different locations during this experiment shows better and promising results as more than 75% of the samples are passed on all the quality parameters. It shows that bio-pesticide available in the market are standardised and effective. In future, it will ensure that agriculture products will be residue free. It will also encourage farmers to export their products as residue free and healthy product will fetch more prices in the international market.

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