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RESEARCH PAPER

Biorational approaches for managing mushroom flies and enhancing yield in oyster mushroom (*Pleurotus eous*) cultivation

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Abstract: Mushroom cultivation, particularly of *Pleurotus eous*, offers nutritional, economic, and environmental benefits but is severely constrained by pest infestations from sciarid (*Bradysia tritici*) and phorid (*Megaselia sandhui*) flies, causing yield losses up to 46%. Overreliance on chemical pesticides poses risks of residues, environmental contamination and pest resistance, highlighting the need for sustainable alternatives. This study evaluated the efficacy of three entomopathogenic fungi (*Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii*) and four botanicals (neem cake, neem oil, eucalyptus leaf extract, tulsi leaf extract) against mushroom flies under Indian cultivation conditions, and assessed their effects on yield and nutritional quality of *P. eous*.Experiments were conducted from November 2020 to January 2021 at JNKVV, Jabalpur, using a completely randomized design with three replications. Treatments were applied at spawning on sterilized wheat–soybean straw substrate. Pest populations (maggots, pupae, adults) were monitored externally and internally at spawn run and across three flushes. Yield (g/bag) and nutritive parameters (protein, ash, carbohydrate, moisture) were determined.Neem cake consistently recorded the lowest sciarid and phorid populations, with maggot and pupal reductions exceeding 65% and adult reductions up to 87% over control. *B. bassiana* performed comparably well among entomopathogens, reducing maggot populations by 31-37% and adults by 39-47%. Tulsi and eucalyptus extracts showed minimal efficacy. Neem cake also achieved the highest yield (797 g/bag; 59.08% over control), followed by *B. bassiana* (778 g/bag; 55.28% over control). Protein (32.22%), carbohydrate (52.40%), and ash content (13.05%) were highest in neem cake treatments, while moisture peaked with eucalyptus extract.The results demonstrate that neem cake and *B. bassiana* offer dual benefits of effective mushroom fly suppression and enhanced yield and nutritional quality. Integrating these biorationals into *P. eous* cultivation can provide a sustainable, pesticide-free approach to pest management and productivity enhancement in mushroom farming.

Key words: Biological control, Entomopathogenic fungi, Mushroom cultivation, Pest management, *Pleurotus eous*.

Introduction

Mushrooms, belonging to the macrofungi group, have long been valued as a nutritious food source, with the Food and Agriculture Organization (FAO) recognizing their superior nutritional quality (Chang and Miles, 2004). Mushrooms, made up of thread-like mycelial structures that develop into fruiting bodies, are believed to have existed long before the emergence of humans. Highly regarded for their taste and health benefits, approximately 2,000 species are identified globally, with 25 species cultivated for consumption. Mushrooms are also considered functional foods for their medicinal properties (Correa *et al*., 2016). Cultivating mushrooms provides economic benefits, especially for underprivileged rural and urban populations, as it requires minimal financial investment and labour. In India, oyster mushrooms, particularly *Pleurotus eous*, have gained popularity for their unique taste, nutritional benefits and rapid growth cycle. Accounting for 16% of the country's total mushroom production, oyster mushrooms thrive on various agricultural waste substrates, such as wheat and rice straw, making them economically and environmentally sustainable (Sharma *et al*., 2017). Despite their potential, India's mushroom production has declined significantly, from 487 million MT in 2017 to 314.84 million MT in 2023 (Anon, 2023). One of the major contributors to this decline is pest infestation. Mushroom cultivation is particularly vulnerable to pests such as phorid flies (*Megaselia sandhui*), sciarid flies (*Bradysia tritici*), mites, and springtails, which can cause significant yield losses ranging from 17 to 46% (Limbule *et al*., 2021). These pests not only directly damage the fruiting bodies but also transmit pathogens and contaminate compost through fecal deposition. Closed cultivation environments exacerbate pest problems, as they provide optimal conditions for their reproduction (Cruz, 2007).While chemical pesticides remain the predominant control method, their use poses challenges such as residue accumulation, environmental harm, and pest resistance (Desneux *et al*., 2007). Increasing consumer demand for pesticide-free produce has highlighted the importance of integrating botanical solutions and biological agents in pest management. Plant-based extracts, functioning as antifeedants and repellents, have emerged as effective tools within Integrated Pest Management (IPM) strategies (Gahukar, 2014). This study focuses on evaluating the efficacy of botanicals and entomopathogens for managing sciarid and phorid flies in

P. eous cultivation under Indian conditions. Additionally, it examines the impact of these biorationals on the nutritional and yield parameters of *P. eous*, offering insights into sustainable pest management and productivity enhancement.

Material and methods

The study investigated the efficacy of different biorationals against mushroom flies infesting *P.eous*. The biorationals included three entomopathogenic fungi—*Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii*—and four botanicals—neem cake, eucalyptus leaf extract, tulsi leaf extract, and neem oil, with water spray as the control. The experiment was conducted at the mushroom production house, Department of Plant Pathology, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh, from November 2020 to January 2021. Seed spawn was sourced from Kerala Agricultural University and a completely randomized design with three replications was followed. A 1:1 ratio of wheat and soybean straw, obtained from the Breeder Seed Production Unit, was used as the substrate. The straw was sterilized by soaking it in water containing 500 ppm formaldehyde and 75 ppm carbendazim for 16 hours (Vijay and Sohi, 1987), followed by draining excess water to achieve 65-70% moisture content. Prior to spawning, formaldehyde was sprinkled on the floor and 3% formaldehyde solution was mixed with the substrate. Bags were filled with 1 kg of substrate mixed with spawn, sealed, perforated for aeration, and incubated until spawn run completion. Once spawn run was achieved, the bags were removed, and the beds were suspended using nylon twines with a 20 cm gap for efficient watering and harvesting. Water was sprayed thrice daily, with watering stopped a day before harvesting.Plant leaf extracts were prepared by grinding equal weights of leaves and water, filtering through muslin cloth and cotton and storing in a refrigerator for future use. Substrates were treated with *M. anisopliae*- 1×10^8 spores/mL (3 mL/lit.), *B. bassiana*-1×10^8 spores/mL (3 mL/lit.), *V. lecanii*- 1 × 10x spores/mL (3 mL/lit.), neem cake (5%), eucalyptus leaf extract (5%), tulsi leaf extract (5%), and neem oil (0.3%) during bagging.The stages of mushroom fly infestation (larval, pupal, and adult) were monitored from spawn run to the third mushroom flush. During the spawn run stage, without removing the polythene bag one observation was taken over the external surface of bag and by removing the polythene bag another observation was taken over beds which was considered as internal count. The observations were taken at spawn run, first flush, second flush and third flush. Mushrooms were harvested over five weeks, with three flushes occurring at 10-12-day intervals. The total yield (kg/bed) was calculated from three flushes. Protein, ash, moisture, and carbohydrate content were analysed as per AOAC (1935). Data were subjected to one-way ANOVA, and Tukey's HSD at 5% significance level was used for comparisons.

Results and discussion

The current study demonstrates the significant impact of biorationals on the population dynamics of mushroom flies and their effect on the yield and nutritive parameters of *P. eous*. The experimental results revealed that treatments with neem cake consistently showed the lowest population of sciarid flies at all stages of mushroom growth. Incorporation of biorationals both externally and internally into mushroom bags reduced maggot and pupal populations significantly, particularly during the spawn run stage. Neem cake application resulted in the least maggot populations, 4.25 and 12.10, during external and internal counts, respectively (Table 1). Among botanicals, tulsi leaf extract showed minimal efficacy, with only a 15.18 and 5.89% population reduction in maggots. Neem cake treatment led to the highest population reduction of pupae, achieving 65.40% and 62.71% reductions. The entomopathogen *B. bassiana* also performed well, with low maggot and adult populations recorded during all three flushes (Fig 1). Neem oil reduced maggot populations by 31.00% -37.00% and adult populations by 39.00% - 47.00%, showing strong efficacy among botanicals.

For phorid flies, the population was generally lower than for sciarids. The least reductions in maggot and pupal populations, 16.83% and 28.77%, were observed in eucalyptus

Table 1. Impact of biorationals on the mushroom flies at spawn run stage of *Pleurotus eous* counted externally and internally

Treatments	External count				Internal count			
	No. of sciarid fly maggot	No. of sciarid fly pupa	No. of phorid fly maggot	No. of phorid fly pupa	No. of sciarid fly maggot	No. of sciarid fly pupa	No. of phorid fly maggot	No. of phorid fly pupa
T1: *Metarhizium anisopilae* - 1×10^8 spores/mL (3 mL/lit.)	7.30 ± 0.11^{d}	7.03 ± 0.11^{d}	4.57 ± 0.07^{de}	4.38 ± 0.07^{c}	18.07 ± 0.28^{c}	17.33 ± 0.26^{c}	8.22 ± 0.13^{d}	8.10 ± 0.12^{d}
T2: *Beauveria bassiana* - 1×10^8 spores/mL (3 mL/lit.)	5.37 ± 0.08^{e}	5.00 ± 0.08^{e}	3.74 ± 0.06^{f}	3.56 ± 0.05^{de}	15.00 ± 0.23^{c}	13.30 ± 0.20^{d}	7.25 ± 0.11^{d}	7.08 ± 0.11^{d}
T3: *Verticillium lecanii*- 1×10^8 spores/mL (3 mL/lit.)	7.12 ± 0.26^{d}	7.07 ± 0.25^{d}	4.10 ± 0.15^{ef}	4.05 ± 0.15^{cd}	17.27 ± 0.62^{cd}	16.38 ± 0.59^{c}	8.00 ± 0.29^{d}	7.59 ± 0.27^{d}
T4: Neem cake (5%)	4.25 ± 0.11^{f}	4.10 ± 0.11^{f}	3.14 ± 0.08^{g}	3.07 ± 0.08^{e}	12.10 ± 0.32^{e}	11.52 ± 0.30^{d}	5.56 ± 0.15^{e}	5.05 ± 0.13^{e}
T5: Eucalyptus leaf extract (5%)	10.33 ± 0.10^{b}	9.30 ± 0.09^{b}	5.24 ± 0.05^{b}	5.20 ± 0.05^{b}	29.10 ± 0.29^{b}	25.62 ± 0.26^{b}	13.20 ± 0.13^{b}	11.64 ± 0.12^{c}
T6: Tulsi leaf extract (5%)	10.73 ± 0.16^{b}	9.77 ± 0.15^{b}	5.14 ± 0.08^{bc}	5.08 ± 0.08^{b}	30.54 ± 0.47^{ab}	30.11 ± 0.46^{a}	13.51 ± 0.21^{b}	12.84 ± 0.20^{b}
T7: Neem oil (0.3%)	8.77 ± 0.28^{c}	8.27 ± 0.27^{c}	4.65 ± 0.15^{cd}	4.30 ± 0.14^{c}	28.50 ± 0.92^{b}	25.27 ± 0.81^{b}	11.75 ± 0.38^{c}	11.65 ± 0.37^{c}
T8: Control (water spary)	12.65 ± 0.26^{a}	11.85 ± 0.25^{a}	6.30 ± 0.13^{a}	7.30 ± 0.15^{a}	32.45 ± 0.68^{a}	32.21 ± 0.67^{a}	14.80 ± 0.31^{a}	14.33 ± 0.30^{a}
C.D. at p=0.05	0.34	0.32	0.41	0.35	0.30	0.29	0.37	0.40

*Means with the same letter are not significantly different from one another at 5% level of significance using Tukey's Honest Significant Difference (HSD) Test *($P < 0.05$)*

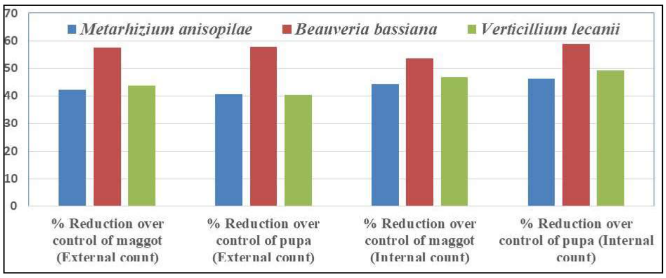

Fig. 1. Effect of the bioagents against the sciarid flies during the external and internal count

leaf extract-treated substrates during external counts. Internal counts for maggots and adults showed population reductions of 8.72% and 10.40%, respectively. Entomopathogens, such as *M. anisopliae* and *V. lecanii*, recorded maggot and adult populations that were statistically on par with each other across the three mushroom flushes. Neem cake-treated bags achieved the highest reductions in maggot and adult populations, ranging from 72.00 to 87.00% over control, consistently outperforming other treatments (Table 2). While entomopathogens were effective in managing mushroom flies, neem cake exhibited superior performance across all stages of mushroom growth.

In addition to reducing mushroom fly populations, biorationals significantly impacted the nutritive parameters of *P. eous*. Neem cake and *B. bassiana* treatments resulted in higher protein content in mushrooms, measuring 32.22 and 30.47%, respectively. Ash content was highest in neem cake-treated bags (13.05%), followed by *B. bassiana* (12.45%). No significant differences in ash content were observed among other treatments. The moisture content of mushrooms was highest in eucalyptus leaf extract-treated bags (85.73%), followed by neem cake. Other treatments ranged between 82.00% and 84.00%. Carbohydrate content was highest in neem cake (52.40%) and neem oil (50.35%), while entomopathogens recorded carbohydrate levels of 48.00% - 49.00%, indicating their nutritional efficacy (Fig 2).

The yield of *P. eous* ranged from 501 g/bag to 797 g/bag (Table 3). The highest yield was observed in neem cake-treated bags, with 797 g/bag and a 59.08% increase over control. Among entomopathogens, *B. bassiana* achieved a yield of 778 g/bag, marking a 55.28% increase over control. *V. lecanii* and *M. anisopliae* performed comparably, with percent increases of 48.90 and 34.13%, respectively. Neem oil-treated bags showed better yields than eucalyptus and tulsi leaf extract-treated bags, despite their lower pest control efficacy. The lowest yield was recorded in tulsi leaf extract-treated bags, which were nearly equivalent to the control.

The study's findings align with previous research emphasizing the effectiveness of biorationals in reducing mushroom fly populations. Neem cake and entomopathogens were particularly effective at preventing flies from laying eggs in treated substrates, as reported by Gahukar (1995) and Pippal (2018). Entomopathogens, such as *B. bassiana*, penetrate insect cuticles, spiracles, and the oral cavity through their hyphae, leading to mortality by spreading blastospores in the insect's circulatory system. This mechanism of action was supported by Andreadis *et al*. (2016). Additionally, the application of entomopathogens did not inhibit mushroom mycelial growth during the spawn run, corroborating findings by Zimmerman (1993).

The higher reduction in pupa populations compared to maggots across treatments was consistent with the observations of Ajvas *et al*. (2020). Neem cake and *B. bassiana*

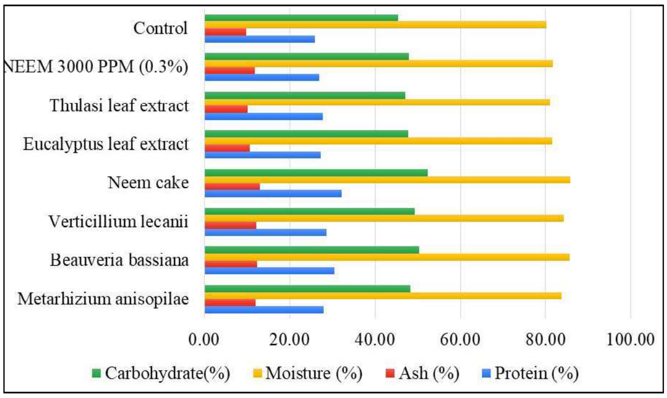

Fig. 2. Impact of biorationals on the nutritive value of *Pleurotus eous*

Table 2. Impact of biorationals on the maggot of mushroom flies at flush stage of *Pleurotus eous*

Treatments	No. of sciarid fly maggot			No. of phorid fly maggot		
	1st flush	2nd flush	3rd flush	1st flush	2nd flush	3rd flush
T1: *Metarhizium anisopilae* -1 × 10⁸ spores/mL (3 mL/lit.)	6.50±0.10^{de}	9.00±0.14^{d}	8.00±0.12^{d}	4.50±0.07^{c}	3.50±0.05^{d}	4.50±0.07^{cd}
T2: *Beauveria bassiana* - 1 × 10⁸ spores/mL (3 mL/lit.)	5.30±0.08^{f}	7.00±0.11^{e}	7.00±0.11^{d}	3.20±0.05^{e}	2.10±0.03^{f}	3.50±0.05^{e}
T3: *Verticillium lecanii*- 1 × 10⁸ spores/mL (3 mL/lit.)	6.20±0.22^{e}	8.10±0.29^{de}	7.50±0.27^{d}	3.75±0.14^{d}	3.00±0.11^{e}	4.00±0.14^{de}
T4: Neem cake (5%)	4.15±0.11^{g}	5.10±0.13^{f}	5.00±0.13^{e}	2.10±0.06^{f}	1.00±0.03^{g}	2.20±0.06^{f}
T5: Eucalyptus leafextract (5%)	8.20±0.08^{c}	12.00±0.12^{c}	10.00±0.10^{c}	5.75±0.06^{b}	5.50±0.05^{b}	6.00±0.06^{b}
T6: Tulsi leaf extract (5%)	9.20±0.14^{b}	13.50±0.21^{b}	11.50±0.18^{b}	6.20±0.09^{b}	4.20±0.06^{c}	6.40±0.10^{b}
T7: Neem oil (0.3%)	7.20±0.23^{d}	11.00±0.35^{c}	9.40±0.30^{c}	4.50±0.14^{c}	3.50±0.11^{d}	4.75±0.15^{c}
T8: Control (water spary)	11.20±0.23^{a}	16.00±0.33^{a}	15.00±0.31^{a}	7.50±0.16^{a}	7.80±0.16^{a}	8.50±0.18^{a}
C.D. at p=0.05	0.41	0.36	0.30	0.31	0.35	0.27

*Means with the same letter are not significantly different from one another at 5% level of significance using Tukey's Honest Significant Difference (HSD) Test *($P < 0.05$)*

Table 3. Impact of the biorationals on the yield of the mushroom

Treatments	Total yield (g/bag)	Percentage increase in yield over control (%)
T1: *Metarhizium anisopilae* -1 × 10^8 spores/mL (3 mL/lit.)	672	34.13
T2: *Beauveria bassiana* - 1 × 10^8 spores/mL (3 mL/lit.)	778	55.28
T3: *Verticillium lecanii*- 1 × 10^8 spores/mL (3 mL/lit.)	746	48.90
T4: Neem cake (5%)	797	59.08
T5: Eucalyptus leaf extract (5%)	582	16.16
T6: Tulsi leaf extract (5%)	543	8.38
T7: Neem oil (0.3%)	614	22.55
T8: Control (water spary)	501	0.00
S.Em±	0.302	
C.D. at 5%	0.912	

showed high efficacy in preventing the maggot stage from developing into pupae or adults. This trend reflects the findings of Gagan *et al.* (2013), Fan *et al.* (2017), and Andreadis *et al.* (2021). The maggot stage is highly vulnerable to entomopathogens and botanicals, allowing for effective control during this stage.

In terms of yield, neem cake and entomopathogens applied at the spawning stage produced significantly higher yields compared to untreated controls or botanicals applied during pinhead initiation or flush intervals. Similar results were reported by Bhat *et al.* (1998), where neem formulations like Neemark and Rakshak improved mushroom yield by 28%-42%. The lignin-degrading enzymes present in oil cakes, including neem cake, accelerate substrate degradation, promoting higher nutrient availability and mushroom growth. This aligns with findings by Hoa *et al.* (2015), Sarker *et al.* (2015), and Singh *et al.* (2020).

Nutritive parameters, such as protein, ash, and carbohydrate content, were also enhanced in neem cake-treated bags, mirroring the results of Bonnati *et al.* (2004), Alam *et al.* (2007), Patil *et al.* (2010), Zied *et al.* (2011), and Chiranjeevi (2020). Moisture content was significantly higher in treated bags, with eucalyptus leaf extract showing the highest levels. Neem cake and neem oil treatments yielded higher carbohydrate content, indicating the suitability of these treatments for enhancing mushroom quality.

Conclusion

From the results of this investigation, it can be concluded that neem cake and the entomopathogenic fungus *Beauveria bassiana*, when applied at the spawning stage of *Pleurotus eous* cultivation, are highly effective in suppressing sciarid and phorid fly populations, with substantial reductions in maggot, pupal, and adult stages compared to untreated control. These treatments also significantly enhanced mushroom yield and nutritive parameters, including protein, carbohydrate, and ash content. Among botanicals, neem cake consistently outperformed others, while eucalyptus and tulsi leaf extracts showed minimal pest suppression. The integration of neem cake or *B. bassiana* into mushroom cultivation practices offers a sustainable, pesticide-free strategy for effective pest management and improved productivity, making them suitable components of Integrated Pest Management (IPM) programmes in mushroom farming.

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