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ASSESSING THE ANTIFUNGAL EFFICACY OF *OCIMUM TENUIFLORUM* ESSENTIAL OIL AGAINST THE PREDOMINANT FUNGAL SPECIES ASSOCIATED WITH MAIZE SEED SAMPLES

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ABSTRACT

Maize (*Zea mays*) is highly vulnerable to fungal contamination during cultivation, storage, and processing, leading to significant economic losses and exposure to mycotoxins. This study evaluates the fungal species associated with maize grains collected from Mirzapur, Varanasi, and Jaunpur district of Uttar Pradesh. The samples were collected from different localities, and the associated mycoflora were isolated and identified using direct plating and serial dilution methods. The isolated fungi were morphologically identified, and the dominant strains were confirmed using standard taxonomic keys. Predominant mycoflora included *Aspergillus flavus, Aspergillus niger, Penicillium glaucum,* and *Fusarium graminearum*. The variation in pH and moisture levels across locations was also noted. Parallely, essential oil was extracted from locally collected plant of *Ocimum tenuiflorum* via hydrodistillation (0.87% v/w). This essential oil was evaluated for antifungal efficacy. The oil exhibited strong activity against all tested fungi, with MIC values ranging from 0.8 to 1.3 µl/ml, where *P. glaucum* was the most sensitive, and *A. niger* was the least sensitive strain among all tested fungi. Ergosterol quantification revealed a dose-dependent decline, with 32-62% reduction at ½ MIC and complete depletion at MIC, indicating severe disruption of the fungal membrane. The results demonstrate the potent antifungal action of *O. tenuiflorum* essential oil and highlight its potential as a green preservative for managing fungal contamination in maize.

***Key words*:** Mycoflora, Essential oil, Antifungal, Ergosterol

Introduction

In many parts of the world, maize (*Zea mays* L.) is the main source of food, feed, and industrial raw materials, making it one of the most significant cereal crops in the world. Its high carbohydrate and amino acid content, as well as versatility in processing, make it central to global food security and livestock nutrition. Maize is widely grown and a vital source of income for farming people in many developing nations, including major agricultural systems in Asia and Africa. However, maize is extremely vulnerable to microbial deterioration during the pre-harvest, post-harvest, and storage phases, even if it has agronomic value (Adiaha *et al*., 2016).

Fungal contamination is a major threat to maize quality and safety. Filamentous fungi such as *Aspergillus flavus, Aspergillus parasiticus, and Penicillium* spp. frequently colonize maize grains under warm and humid conditions. These pathogens not only reduce nutritional and germination quality but also produce toxic secondary metabolites, including aflatoxins and fumonisins, which pose severe risks to human and animal health (Ekwomadu *et al*., 2018). Aflatoxin B_1, in particular, is considered one of the most potent naturally occurring carcinogens, and its presence in maize leads to significant economic losses due to strict regulatory limits and rejected consignments. Moreover, contamination often begins in the field under abiotic stress conditions such as drought, insect damage, or improper agronomic practices, which predispose maize ears to fungal invasion. The problem is further amplified during post-harvest stages, where inadequate drying, poor storage infrastructure, and fluctuating temperature or humidity levels facilitate fungal proliferation (Yohannis *et al*., 2025). Once established,

fungi can remain active throughout storage, causing progressive deterioration and continued mycotoxin accumulation. This persistent contamination cycle poses a significant challenge to food safety systems, especially in regions lacking robust monitoring and control mechanisms.

In recent years, essential oils (EOs) derived from aromatic plants have emerged as promising eco-friendly alternatives for managing fungal contamination in food and feed systems. Rich in bioactive terpenes and phenolic compounds, EOs exhibit strong antifungal and antiaflatoxigenic properties. Their natural origin, biodegradability, and compatibility with green preservation strategies make essential oils attractive candidates for replacing or supplementing synthetic fungicides in maize protection (Liang *et al*., 2023). *Ocimum tenuiflorum* essential oil (EO), commonly known as tulsi oil, is rich in bioactive components such as eugenol, methyl eugenol, caryophyllene, and linalool. The oil exhibits notable antifungal activity against storage pathogens. Owing to its safety, biodegradability, and potent efficacy, *O. tenuiflorum* EO is increasingly considered a viable component in sustainable grain protection strategies.

Materials and Methods

Isolation of Fungi from Maize Seeds

Maize samples were obtained from small-scale subsistence farmers in Varanasi, Jaunpur, and Mirzapur districts of Uttar Pradesh, India. The samples were stored in pre-sterilized polyethylene bags to prevent contamination and kept at 4°C until further analysis. The moisture content and pH of maize seeds were determined following the method described by Prakash *et al*., (2012). For fungal isolation, maize seeds were surface sterilized with 1% sodium hypochlorite for 1–2 minutes, rinsed with sterile distilled water, and air-dried under aseptic conditions (Sahu *et al*., 2022 & Govender *et al*., 2008). Seeds were then plated as direct plating on Potato Dextrose Agar (PDA) medium and incubated at 27 ± 2 °C for 5-7 days. Further, 1 g of each sample was crushed and serially diluted and spread on the PDA plates; The number of fun gal colonies was counted after incubation at 27 ± 2 °C for 5–7 days. Emerging fungal colonies were sub-cultured on fresh PDA plates to obtain pure cultures.

Morphological Characterization of Identified Fungi

Several fungal species commonly associated with maize under pre-harvest and storage conditions were identified, including the predominant species *Aspergillus flavus, Aspergillus niger, Penicillium glaucum*, and *Fusarium graminearum*. These fungi were isolated from naturally infected maize grains and characterized based on colony morphology and microscopic features. Morphological characteristics were examined using a binocular microscope fitted with a digital camera. Microslides were prepared using lactophenol-cotton blue staining for detailed observation of fungal structures, including Stromata, Conidia, Conidiophores, Hyphal morphology, and Colony colour and texture (Abhinav *et al*., 2026). Identification of fungal isolates was performed using standard taxonomic keys and the manual "A Manual of Soil Fungi" By Joseph C. Gilman.

Selection and identification of *Ocimum tenuiflorum* for the extraction of essential oil

Fresh leaves and tender aerial parts of healthy plants of *Ocimum tenuiflorum* L. (syn. *Ocimum sanctum*), also referred to as holy basil, Tulsi, or Holy Basil, were collected from Banaras Hindu University, Varanasi, Uttar Pradesh, India. *Ocimum tenuiflorum* is an aromatic perennial shrub, a member of the family Lamiaceae, characterized by quadrangular stems, opposite ovate leaves with serrated margins, glandular trichomes, and a strong clove-like aroma attributed to eugenol-rich essential oil. The terminal racemes of flowers, which are usually white or purple in colour, are crucial morphological characteristics for identification using regional floras and standard botanical keys.

Before extracting the essential oils, the collected plant material was shade-dried at room temperature to

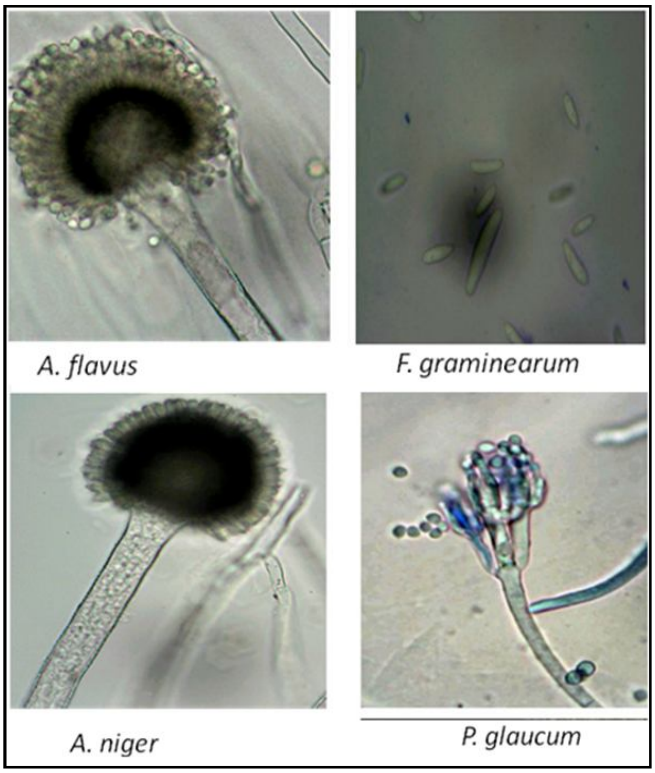

Fig. 1: Microscopic identification of isolated fungal species.

Table1: Mycoflora analysis of *Zea mays* seed samples.

Location	Fungal species						pH	Moisture Content (%)
	a.f	a.n	p.g	a.a	f.g	c.l		
***Zea mays* (Mirzapur)**	64	37	25	07	12	11	6.4 ± 0.02^{a}	13.2 ± 0.10^{a}
***Zea mays* (Varanasi)**	43	18	39	12	26	00	6.1 ± 0.03^{b}	12.5 ± 0.12^{b}
***Zea mays* (Jaunpur)**	72	32	36	02	28	05	5.9 ± 0.01^{c}	11.8 ± 0.08^{c}
Note: a.f. *Aspergillus flavus*, a.n *Aspergillus niger*, p.g *Penicillium glaucum*, a.a *Alternaria alternata*, f.g *Fusarium graminearum*, c.l *Cladosporium lunata*								

preserve its volatile contents and cleaned with distilled water to get rid of dust and debris. The species' high essential oil concentration in leaves and flowering tops, especially the presence of eugenol, methyl eugenol, and other phenolic compounds, as well as its traditional medicinal value, led to its selection for the current study (Prakash & Gupta, 2005; Pandey & Madhuri, 2010). The plant parts were added to the hydrodistillation unit along with water and were heated at 90°C for 4 hours (Raghuvanshi *et al.*, 2026). The water droplets were removed by adding 5 mg of sodium sulphate to the collected oil sample and stored at room temperature.

Antifungal efficacy of isolated essential oil against selected fungi

The antifungal activity of EO against the chosen fungi (*Aspergillus flavus, A. niger, Fusarium grameniarum, and Penicillium glaucum*) was examined using a poisoned-food technique as outlined by Shukla *et al.*, (2012). The mycelial disc of seven-day-old fungi cultivated on PDA media was briefly removed from the edge, measuring 5 mm. Each fungal mycelial disc was then placed in a Petri plate containing PDA media with the appropriate EO concentration (0.2 to 1.5 µL/mL) and maintained at 27±2°C in a BOD incubator. When there was no visible development on a Petri plate after 10 days of incubation, the MIC was noted with a colony diameter of less than 1 mm, which is considered no visible growth.

Effect of different doses of essential oil on ergosterol content

The method of Kumar *et al.*, (2024) was used to quantify the ergosterol from the test fungus. For five days, a 5mm disc containing a five-day-old colony of each fungus was placed in fungal growth media SMKY. The mycelia were then extracted from each sample independently. The mycelia were weighed, then moved to a 5 mL alcoholic KOH (25%) solution and carefully vortexed. After two hours at 80°C in a water bath, each sample solution was vortexed once more for five minutes and exposed to five millilitres of sterile water and n-heptane 1:3. The following formula was used to quantify ergosterol in the resultant supernatant, which contains an opaque coating of n-heptane:

$$\text{Ergosterol (\%)} = (A_{282/290} - A_{230/518})/W$$

A: Absorbance at 282 and 230 nm; 290 and 518= extinction coefficient of ergosterol and dehydroergosterol

Statistical Analysis

All experiment in the present study was done in triplicate, and data are reported as the mean ± SE.

Result and Discussion

Extraction and isolation of fungal cultures from maize

The fungal species were obtained by growing the maize seed collected from different locations on PDA plates by direct plating and serial dilution methods. The number of colonies of each fungal species was counted and maintained in a table along with pH and moisture (Table 1). Each fungus was then isolated on separate plates and recultured to obtain a pure culture.

Morphological identification of dominant fungi

The pure isolates of fungal cultures were identified using "A Manual of Soil Fungi" By Joseph C. Gilman, based on their morphological and microscopic characteristics.

Aspergillus flavus typically produces rapidly growing colonies that appear yellow-green to olive on potato

Fig. 2: *Ocimum tenuiflorum* plant.

dextrose agar (PDA). The mycelium is hyaline and septate, and conidiophores arise from specialized foot cells. The vesicles are globose to sub-globose and bear phialides arranged in uniseriate or biseriate form. Conidia are spherical, rough-walled, and produced in radiating chains, forming characteristic conidial heads. Sclerotia may also be present in some strains, serving as survival structures (Klich, 2002; Pitt & Hocking, 2009).

Aspergillus niger forms fast-growing colonies that are initially white and later turn black due to abundant conidial production. Microscopically, it shows long, smooth, hyaline conidiophores terminating in globose vesicles covered with biseriate phialides. The conidia are dark, globose, and rough-walled, forming large radiate conidial heads. Septate hyphae and a well-developed mycelial network are typical features used for identification (Pitt & Hocking, 2009).

Penicillium glaucum produces velvety colonies that are typically blue-green to green in colour with a white margin. The vegetative hyphae are hyaline and septate. The conidiophores branch to form a characteristic "penicillus" (brush-like structure), consisting of metulae and phialides that produce chains of globose conidia. These conidia are usually smooth to slightly roughened and form dry spore masses responsible for the powdery colony appearance. The penicillus arrangement is a key diagnostic feature of the genus *Penicillium* (Parveen *et al.*, 2017)

Fusarium graminearum produces rapidly growing colonies with dense mycelium that ranges from white to pale orange or yellow on PDA. The fungus forms sporodochia that are orange to reddish-brown. Microscopically, it produces slender, sickle-shaped macroconidia with typically five to six septa, a pointed apical cell, and a foot-shaped basal cell. Chlamydospores may form in chains or clusters, and the species can produce perithecia under suitable conditions. These morphological features are diagnostic for species identification within the genus *Fusarium*. (Ramos *et al.*, 2014).

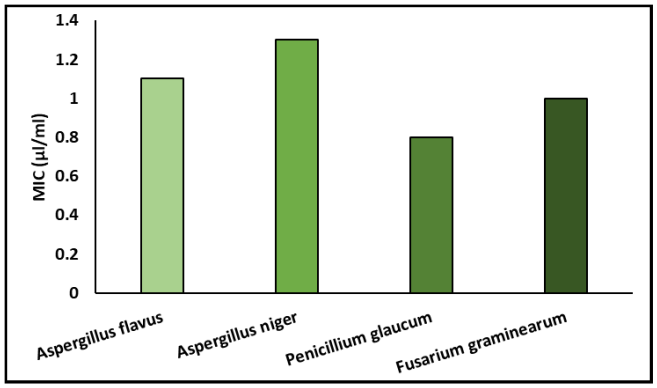

Fig. 3: MIC of *O. tenuiflorum* essential oil against selected fungal strains.

Extraction of essential oil from *Ocimum tenuiflorum*

Ocimum tenuiflorum plant is widely acknowledged as a medicinally valuable species in Ayurveda and traditional medicinal systems, owing to its rich phytochemical composition, including essential oils, flavonoids, phenolic compounds, and terpenoids, which contribute to its diverse therapeutic properties. *O. tenuiflorum* exhibits antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and adaptogenic properties that highlight its significance in herbal medicine, nutraceutical preparations, and essential-oil-based formulations, as well as its growing relevance in modern pharmacological research and natural product development (Sahu, 2025). The essential oil extracted from aerial plant parts by hydrodistillation resulted on yield of 0.87% v/w. The oil was stored in an amber bottle at room temperature for further experiments.

Antifungal activity of the extracted essential oil against selected fungal strains

The minimum inhibitory concentration (MIC) of *Ocimum tenuiflorum* essential oil varied significantly among the four fungal pathogens evaluated (*Aspergillus flavus, Aspergillus niger, Penicillium glaucum, and Fusarium graminearum*). *P. glaucum* showed the maximum sensitivity to the essential oil, with the lowest MIC value of 0.8 µl/ml. *F. graminearum* demonstrated moderate susceptibility, requiring 1.0 µl/ml for complete growth inhibition. *A. flavus* exhibited an MIC of 1.1 µl/ml, followed by *A. niger*, showing the highest MIC value of 1.3 µl/ml.

This antifungal activity of EO against the tested fungi highlights its promising bioactive potential. The lower MIC values for *P. glaucum* and *F. graminearum* suggest higher vulnerability of these species to the EO, which commonly includes eugenol, methyl eugenol,

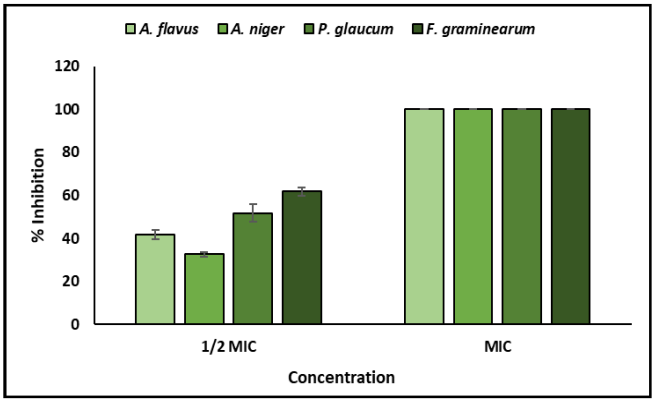

Fig. 4: Effect of *O. tenuiflorum* essential oil treatment on ergosterol content.

caryophyllene, and other monoterpenes, which are known for their membrane-disruptive activities. The pronounced sensitivity of *P. glaucum* (0.8 µl/ml) could be linked to its relatively less rigid cell wall structure. In contrast, *A. niger* exhibited the highest MIC (1.3 µl/ml), signifying a stronger tolerance to the EO treatment. This resistance may stem from its thicker cell wall and more efficient detoxification, which can collectively reduce intracellular accumulation of essential oil components (Osset-Trénor *et al*., 2023).

Assessment of ergosterol content at half MIC and MIC dose

The effect of *Ocimum tenuiflorum* essential oil on ergosterol content in the tested fungi was evaluated at ½ MIC and MIC concentrations. A dose- dependent decline in ergosterol levels was observed across all four fungal strains. At ½ MIC, the essential oil caused a moderate reduction in ergosterol, with inhibition ranging from 32 to 62%. *A. niger* showed the lowest decrease (32.66%), followed by *A. flavus* (41.80%), whereas *P. glaucum* and *F. graminearum* exhibited more substantial reductions of 51.77% and 61.72%, respectively. At the MIC concentration, ergosterol content dropped sharply, reaching complete inhibition in all fungi.

Ergosterol is a key structural component of fungal cell membranes, and its depletion indicates severe disruption of membrane integrity and impaired sterol biosynthesis (Choy *et al*., 2023). The observed concentration-dependent decline in ergosterol content demonstrates that *O. tenuiflorum* essential oil can interfere with sterol synthesis pathways.

The moderate but significant reduction in ergosterol at ½ MIC suggests that even sub-inhibitory levels of the essential oil compromise membrane organization and fluidity, weakening fungal cellular functions. The greater reduction in *P. glaucum and F. graminearum* compared to *A. flavus and A. niger* implies species-specific susceptibility. *A. niger*, showing the least reduction, can be attributed to its comparatively rigid cell wall and robust membrane repair systems. At MIC, the complete depletion of ergosterol across all fungi demonstrates a strong inhibitory action. The collapse of ergosterol content at MIC levels correlates with the 100% growth inhibition observed, reinforcing the role of membrane disruption in antifungal activity.

Conclusion

Maize samples collected from different locations were contaminated with a variety of fungi. The analysis of the mycoflora revealed that the contamination was dominated by *Aspergillus flavus, Aspergillus niger, Penicillium glaucum,* and *Fusarium graminearum*. Essential oil extracted from *Ocimum tenuiflorum* exhibited significant antifungal activity against isolated fungi. Its strong inhibitory effects, coupled with complete ergosterol depletion at MIC, confirm membrane disruption as a key mechanism of action. The EO attributed to the highest efficacy against *P. glaucum* and *F. graminearum*, indicating species-specific susceptibility.

Thus, *O. tenuiflorum* essential oil could be used as a promising green alternative for preventing fungal spoilage in stored maize.

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References

Abhinav, Naik A.K., Rajwar S., Singh G., Singh R. and Kumar S. (2026). First report of Corynespora cassiicola causing shot-hole diseases in Mesosphaerum suaveolens in India based on polyphasic evidence. *Physiological and Molecular Plant Pathology*, **142**, 103112.

Adiaha, M.S., Agba O.A., Attoe E.E., Ojikpong T.O., Kekong M.A., Obio A. and Undie U.L. (2016). Effect of maize (*Zea mays* L.) on human development and the future of man-maize survival: A review. *World Scientific News*, **59**, 52-62.

Choy, H.L., Gaylord E.A. and Doering T.L. (2023). Ergosterol distribution controls surface structure formation and fungal pathogenicity. *MBio*, **14(4)**, e01353-23.

Ekwomadu, T.I., Gopane R.E. and Mwanza M. (2018). Occurrence of filamentous fungi in maize destined for human consumption in South Africa. *Food science & nutrition*, **6(4)**, 884-890.

Gilman, J.C. and Joseph C. (1998). *A manual of soil fungi*. Daya Books.

Govender, V., Aveling T.A.S. and Kritzinger Q. (2008). The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique. South African Journal of Botany, **74(2)**, 190–196.

Klich, M.A. (2002). *Identification of common Aspergillus species*. Utrecht, Netherlands: Centraalbureau voor Schimmelcultures (CBS).

Liang, H., Yang M., Li Q., Zhang L. and Zhao X. (2024). A comprehensive review of the main components of plant essential oils and the mechanisms responsible for the inhibitory effects on fungal growth and aflatoxin synthesis. *Innovative Food Science & Emerging Technologies*, **96**, 103747.

Omm Sahu (2025). A Comprehensive Review on Ocimum

tenuiflorum. *Journal of Ayurveda and Integrated Medical Sciences*, **10(7)**, 193–200.

Osset-Trénor, P., Pascual-Ahuir A. and Proft M. (2023). Fungal drug response and antimicrobial resistance. *Journal of Fungi*, **9(5)**, 565.

Pandey, Govind and Madhuri Sharma (2010). Pharmacological activities of Ocimum sanctum (*Tulsi*): A review. *International Journal of Pharmaceutical Sciences Review and Research*. **5**. 61-66.

Parveen, S., Wani A.H., Bhat M.Y., Wani T.A. and Malik A.R. (2017). First report of Penicillium glaucum Link causing Penicillium rot of pear fruits, Pyrus communis L. in Jammu and Kashmir, India. *Brazilian Journal of Biological Sciences*, **4(8)**, e275.

Pitt, J.I. and Hocking A.D. (2009). *Fungi and food spoilage* (3rd ed.). Springer, New York.

Prakash, P. and Gupta N. (2005). Therapeutic uses of Ocimum sanctum Linn (*Tulsi*) with a note on eugenol and its pharmacological actions: a short review. *Indian J Physiol Pharmacol*. **Apr;49(2)**, 125-31. PMID: 16170979.

Prakash, B., Singh P., Kedia A. and Dubey N.K. (2012). Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities, and in vivo efficacy in the food system. *Food Research International*, **49(1)**, 201–208.

Raghuvanshi, T.S., Gupta V. and Prakash B. (2026). Assessing the antibacterial, antifungal, and antiaflatoxigenic activity of a developed nanoformulation based on a combination of Syzygium aromaticum leaf and bud essential oils. *Sustainable Food Technology*.

Ramos, D.P. and Barbosa, Rafael, Vieira B.G.T.L., Panizzi, R. and Vieira Roberval. (2014). Fusarium graminearum and Fusarium verticillioides infection on maize seeds. *Pesquisa Agropecuaria Tropical*. **44**, 24-31.

Sahu, P.K., Tilgam J., Mishra Sushma, Hamid S., Gupta A.K., Verma S.K. and Kharwar R.N. (2022). Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *Journal of Basic Microbiology*, **62(6)**, 647–668.

Yohannis, E., Urugo M.M., Teka T.A., Getachew P., Tola Y.B., Forsido S.F. and Teferra T.F. (2025). Aflatoxin Contamination in Agri Food Systems: A Comprehensive Review of Toxicity, Food Security, Economic Impacts, and Sustainable Mitigation Across the Value Chain. *Food Science & Nutrition*, **13(10)**, e71104.