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COLLECTION, ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF *Beauveria bassiana* ISOLATES

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ABSTRACT

The investigation was conducted at Bilaspur division of Chhattsgarh during the *kharif* and *rabi* seasons of 2023-24 with the aim to collect new indigenous isolates of entomopathogenic fungi and to characterizes *Beavaria bassiana*, morphologically for identification and to know the variation amongst the isolates . Identified eight isolates of *B. bassiana*, the isolates BV_1, BV_2, BV_4 and BV_8 showed raised cottony colony growth with raised centre. The upper surface of the colony showed white colour while lower surface was pale cream to pale dark cream. Whereas isolates BV_3 showed flat cottony light pink with pale cream and BV_7 showed light pink with pale dark cream colour in upper and lower surface. Isolates BV_5 and BV_6 were raised cottony colony growth with light pale cream to pale cream in upper and lower surface.The isolates of *B. bassiana* showed different textures, colony colors and slightly variation in length (2.05-2.66 µm) and width (1.29-2.24 µm) and shape of conidia. Mostly *B. bassiana* conidia was oval in shape with hyaline in colour.

(Key words: Entomopathogenic fungi, *Beavaria bassiana*, morphological, cultural characters)

INTRODUCTION

Entomopathogens that are harmful to many insects of various orders and effectively manage the inputs like *Beauveria bassiana* are well-characterized Sordariomycetes fungus that is found worldwide and primarily affects Lepidopterian insects. It is a member of the Hypocereals order. Mostly entomopathogenic fungi especially *Beauveria bassiana* was used as biological control agents of insects. Classified under the genus *Botrytis* which is also responsible for the muscardine sickness that affects silkworms (Beauverie, 1914). After *Beauverie*, Vuillemin (1912) named and combined the genus *Beauveria* for the first time, designating *bassiana* as the type species. The entomopathogenic fungi have great potential to limit the feeding capacity of many insects by producing toxic compounds that distress the insects. These are more effective and promising than chemical due to pesticide resistance and resurgence. (Nirmalkar *et al*., 2020)

Collection, isolation and characterization of *B. bassiana* are quit importance because the entomopathogenic fungi are effective against wide range of insect's pests and orders that's way the investigation was taken for selection of most virulent isolates of entomopathogens.

MATERIALS AND METHODS

Collection and isolation of *B. bassiana* isolates through insect cadavers

Naturally infected insect's cadavers were collected from different cultivated fields in different locations of Bilaspur division. Dead insect's were brought to the laboratory for examination. Insects were surface sterilized in the solution of 1% sodium hypochlorite and then rinsed with sterile water. Insects were placed in petri dishes lined with moistened filter paper and kept in the BOD for 24 hours at 25± 2 °C. The insects were observed daily for fungi emerging on the surfaces of the cadavers infected by *B. bassiana*. The fungi were isolated directly from dead insects by transferring external conidia from dead insects into potato dextrose agar (PDA) medium amended and incubated at 25 ±2°C for further growth and subsequent sub culturing.

Collection and isolation of *B. bassiana* isolates through soil baiting method

Soil samples were collected from different locations of Bilaspur division from cultivated fields, standard soil sample procedure was followed. The Galleria bait method was used to isolate EPF from the soil samples. Greater wax moth larvae (*Galleria mellonella L.*) are susceptible to infection by entomopathogenic fungi. The larvae were used as bait insect's. The collected soil samples were sieved,

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moistened with sterile water and transferred to aluminum container and then baited with the wax moth larvae. Five larvae of 3rd instar were placed into the container with sterile forceps and incubated at 25±2^{0}C. Containers were turned upside down daily for 7-10 days. Dead larvae were removed from the soil with sterile forceps and placed in a 1% sodium hypochlorite solution for 3 min for surface sterilization of larvae, after which they were washed in sterile distilled water for 3 min. Excess water was removed by dabbing with a piece of sterilized paper and the dead larvae were placed individually in sterilized petri dishes on humid filter paper. The petri dishes were incubated at 25±2^{0}C and checked for fungal outgrowth every 2 days. When the presence of a fungus was observed, conidia were removed from the insect surface with a sterile loop and streaked in potato dextrose agar plates.

Purification of fungi

One to three days after incubation in PDA (potato dextrose agar) medium the fungus was purified by sub-culturing and slant of purified culture were prepared

Morphological characterization

Insect-harming fungus *viz., Beauveria bassiana* was culturally examined based on their colony characters, *i.e.,* colony texture, colony colour, spore shape, colony pigmentation, spore colour, spore size and growth pattern on potato dextrose agar medium. The fungi were grown on PDA allowed to evaluate colony characters at the 7th DAI for *B. bassiana*. A tiny thread from a pure culture of *B. bassiana* of 15 days old was taken on glass slides with a sterile needle for cultural and morphometric studies. Colony texture and colony colour of EPF were recorded at 8th Days after inoculation. While for spore a small amount of 15th day old pure culture of EPF (*B. bassiana*) was taken on glass slide with a sterile needle and observed under microscope. The mycelium was then examined under a microscope. Spores were measured under compound microscope at 40x magnification after the ocular micrometer was calibrated with a stage micrometre. The presence of conidiophores and phialides two similar exterior morphological features was noted as per formula given by Nirmalkar (2000).

$$\text{One division of ocular micrometer } (\mu) = \frac{\text{No. of division of stage micrometer}}{\text{No. of division of ocular micrometer}} \times 10$$

Size = Calibration factor x No. of division of ocular micrometer

RESULTS AND DISCUSSION

Collection and isolation of *B. bassiana* isolates from insect cadavers and soils

Insect cadavers were collected from various location of Bilaspur division and collected 44 insect cadavers from different crops i.e. groundnut (*Spodoptera litura*), Urd (*Helicoverpa armigera* , soybean (*H.armigera*), moong (*H.armigera*), rice (*Nephotettix nigropictus*), pigeonpea (*H.armigera*) , tomato (*Thrips tabaci*). Amongst all cadavers seven species of *B. bassiana* was isolated from Tobacco caterpillar (*Spodoptera litura*). During the process of isolation different others pathogen i.e. *Tricoderma*, *Aspergillus*, *Fusarium* were isolated from insect cadavers which was lesser pathogenic or non pathogenic to insect and cannot consider for further study. Various twenty five soil samples were collected from different location of Bilaspur division of Chhattisgarh and were used for Galleria baiting method. Among all collected soil samples one spp. of *B. bassiana* was identified. Different workers also reported entomopathogenic fungi by larva of *Spodoptera litura* and others. *B. bassiana* isolated from *Spodoptera litura* from groundnut crops. Nirmalkar *et al*. (2020) also reported similar findings and isolated entomopathogenic fungi from insect cadavers of different crops i.e. Soybean, groundnut and pigeonpea. Thakur and Sandhu (2010) also reported similar finding, they isolated entomopathogenic fungi i.e. *Nomeraea riley* and *Paecilomyces fumosoroseus* and were identified in naturally infected insects i.e. *Hyblaea puera* (teak defoliator), *Eutectona machaeral* (teak skeletonizer) and *Spodoptera litura* (tobacco cutworm).

B. bassiana was isolated from different location of soil by Galleria bait method. Sookar *et al*. (2008) and Baydar *et al*. (2016) isolated entomopathogenic fungi *B. bassiana* from galleria bait method. Sabbour *et al.* (2011) also found *B. bassiana* from soil and tested against a variety of corn borer insect pest.

Morphological characterization

The morphological characterizations of different isolates (BV_1, BV_2, BV_4 and BV_8) of *B. bassiana* showed different colony textures mostly they showed raised cottony growth with raised center. The upper surface of the plates showed white in colour while lower surface of plates were pale cream to pale dark cream in colour. Conidia were oval in shape and hyaline in colour, length and width ranged from 1.92- 2.66 µm and 1.38- 2.24 µm respectively. Whereas the colony texture of BV_3 and BV_7 showed flat cottony to flat cottony light pink with pale cream to pale dark cream colour in upper and lower surface, respectively while, the conidial length and width were in the range of 2.10-2.30 µm to 1.26-1.38 µm in size and conidia were oval in shape and hyaline in colour. Similarly, isolates BV_5 and BV_6 showed raised cottony texture with light pale cream to pale cream colour in upper and lower surface, respectively and conidial length and width ranged from 2.05-2.27 µm to 1.29-1.58 µm in size with oval in shape and hyaline in colour. (Table 1).Conidiogenous cells of *B. bassiana* were subspherical to ampulli form 3-6 µm, phialides hyaline, solitary, smooth walled and aseptate hyphae (plate 1).

Based on cultural and morphological characters the fungi was systemically classified as:- It belongs to Division – Ascomycota, Class – Sordariomycetes, Order – Hypocereals, Family – Cordycipitacea, Genus – *Beauveria*, Species – *bassiana.* Similar findings were also brought by several researchers. Humber (2012) characterized fungal morphology and found pale to yellow colony color, globose or sub globose shape of conidia, white to cream color of conidia spores. Kulu *et al. (*2015) also done morphological

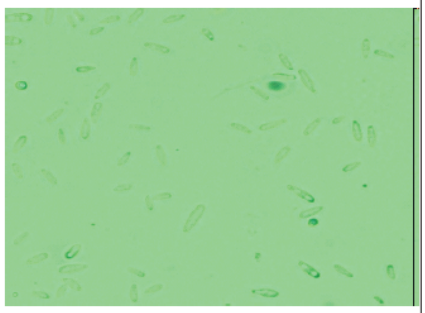
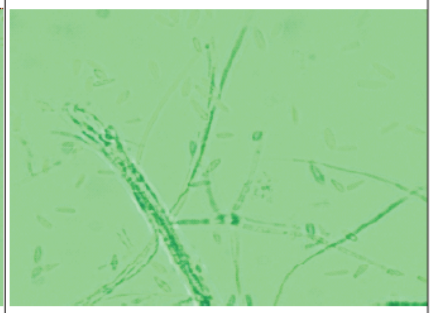
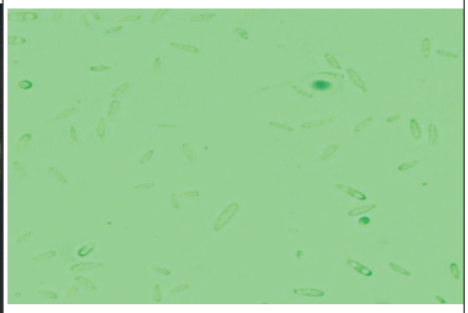
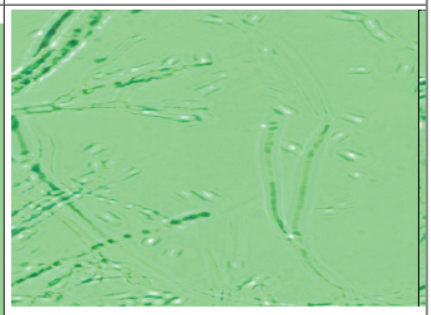
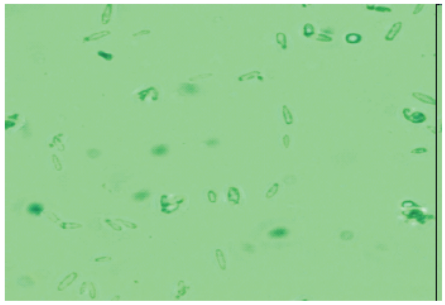
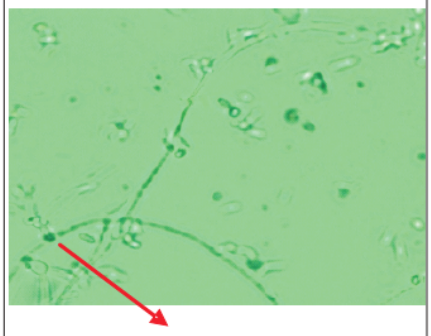
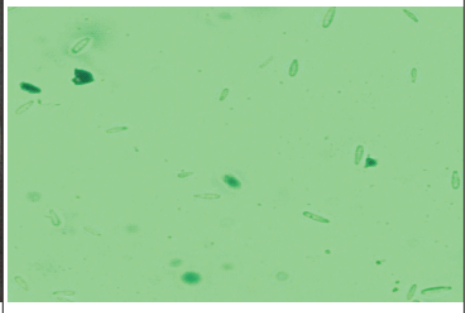
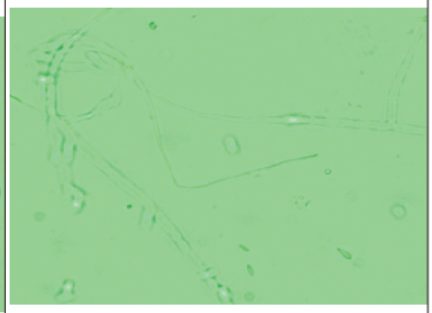
Table 1. Details of entomopathogenic fungi *B. bassiana* isolated from insect cadaver's and soil

Sr. No.	Fungi identified	Source	Code
1	*B. bassiana*	*Spodoptera litura*	BV_1
2	*B. bassiana*	*Spodoptera litura*	BV_2
3	*B. bassiana*	*Spodoptera litura*	BV_3
4	*B. bassiana*	*Spodoptera litura*	BV_4
5	*B. bassiana*	*Spodoptera litura*	BV_5
6	*B. bassiana*	*Spodoptera litura*	BV_6
7	*B. bassiana*	*Spodoptera litura*	BV_7
8	*B. bassiana*	Soil	BV_8

Table 2. Morphological characterizations of entomopathogenic fungi *B. bassiana*

Sr. No.	Isolates	Colony texture	Colony colour		Shape and colour of conidia	Size of conidia	
			Upper surface	Lower surface		Length (µm)	Width (µm)
1	BV_1	Raised cottony with raised centre	White	Pale dark cream	Oval hyaline	1.92	1.53
2	BV_2	Raised cottony with raised centre	White	Pale cream	Oval hyaline	2.59	1.56
3	BV_3	Flat cottony	Light pink	Light pale cream	Oval hyaline	2.10	1.38
4	BV_4	Raised cottony with raised centre	White	Pale cream	Oval hyaline	2.49	1.43
5	BV_5	Raised cottony	White	Light pale cream	Oval hyaline	2.27	1.29
6	BV_6	Raised cottony	White	Pale cream	Oval hyaline	2.05	1.58
7	BV_7	Flat cottony light pink	Light pink	Pale dark cream	Oval hyaline	2.30	1.26
8	BV_8	Raised cottony with raised centre	White	Pale cream	Oval hyaline	2.66	2.24

BV- *Beauveria bassiana* , µm- micrometer

Isolates	Conidia	Conidiophore
 BV_1		
 BV_2		
 BV_3		 Phialides
 BV_4		

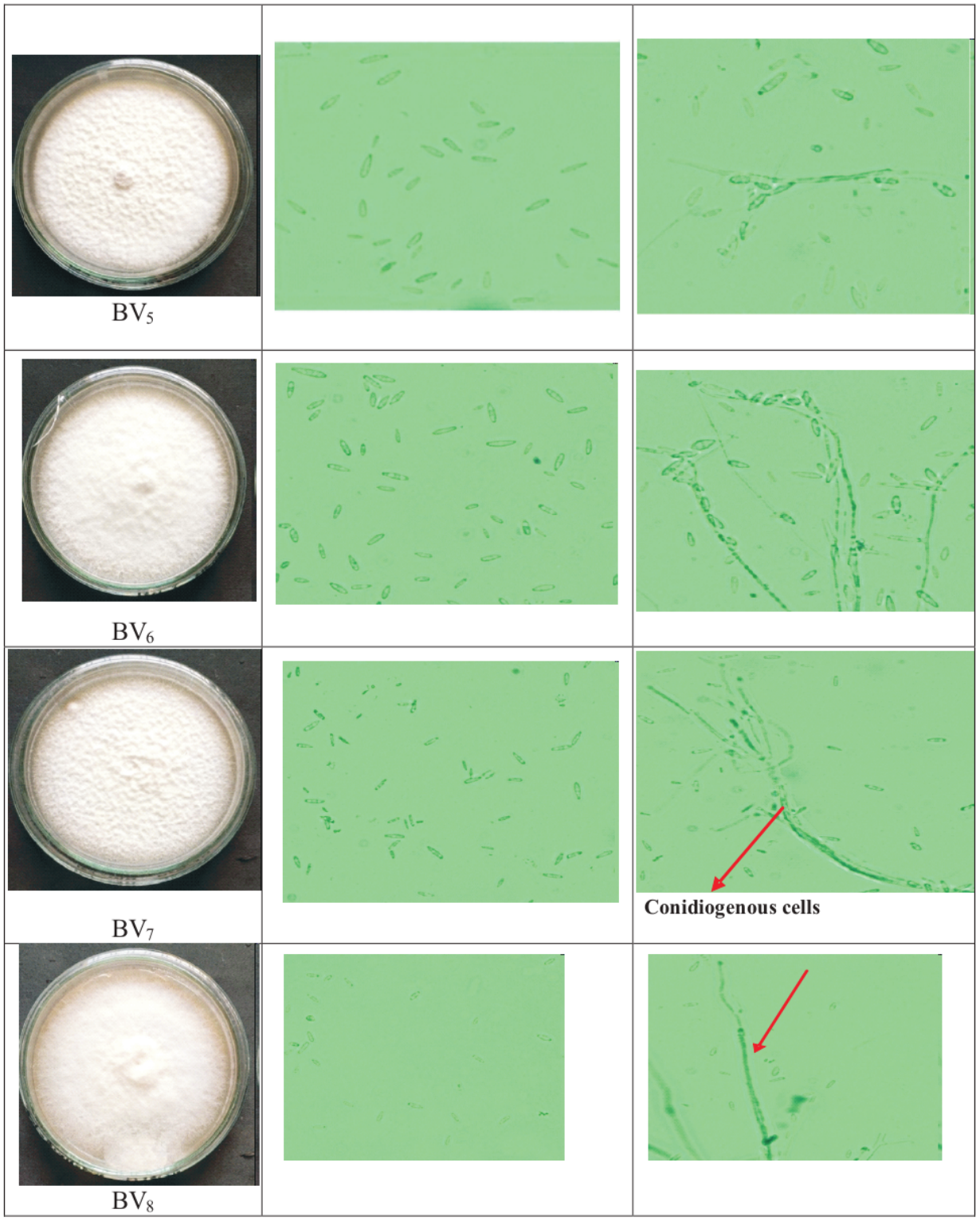


Plate 1. Colony characteristics on potato dextrose agar medium of different isolates of *B. bassiana* and Conidia and conidiogenous cells, phialides arrangement of *B. Bassiana* (BV_1-BV_8) at 40X.

characterization of *B. bassiana* isolates and found white colony color, texture was smooth as like powder and it was identical when colony growth more. Verma *et al*. (2023) also uses the similar media for morphological characterization of *Paecilomyces spp.* and Norjmaa *et al.* (2019) observed the similar based on microscopic observation, as per their observation hyphae have branched and formed conidiogenous cells and long branch. Single cell *B. bassiana* conidium was round and tends to oval.

The variation between the isolates was seen due to the agroecological variation from these isolates. The agroecological situation was differ in terms of rainfall, temperature, humidity, cold, heat etc. That's why the morphological variation among the isolates was observed.

For collection and isolation of *B. bassiana* targets the lepidopteron insects mainly *Spodoptera* sp. and collected soils samples from naturally cultivated fields microscopic study was better for preliminary identification of *B. bassiana*. But it needs molecular identification of isolates for confirmation as per international rules and classification.

REFERENCES

Baydar, R., R. Guven and I. Karaca, 2016. Occurrence of entomopathogenic fungi in agriculture soil from Isparta Province in Turkey and their pathogenicity to *Galleria mellonella* (Lapidoptera : Pyralidae) larvae. Egy. J. boil. Pest cont. **26**(2): 323-327.

Beauverie, M.I. 1914. Les. Muscardines Ie genre *Beauveria* Vuillemin. Revue Gen. Bot. **26**: 81-157.

Humber, R. A. 2012. Manual of Techniques in Invertebrate Pathology. Second edition, Academic press An imprint of Elsevier Science Amsterdam / Boston / Heidelberg / London / New York Oxford / Paris / San Diego /San Francisco / Sydney /Tokyo. pp. 42.

Kulu, I.P., A.L Abadi , A. Afandhi and N. Aidawati , 2015. Morphological and Molecular Identification of *Beauveria bassiana* as Entomopathogen Agent from Central Kalimantan Peatland Indonesia. Int. Chem. Tech. Res. **8**:2079-2084.

Nirmalkar, V.K. 2020. Characterization and efficacy of entomopathogenic fungi collected from different parts of Chhattisgarh. Unpublished PhD Thesis, Indira Gandhi Krishi Vishwavidyala, Raipur.

Nirmalkar, V.K., R.K .S. Tiwari and N. Lakpale, 2020. Efficacy of different carbon and nitrogen sources against mycelial growth and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae*. J. Soils and Crops. **30** (2): 206-212.

Norjmaa, U., D. Nasandulam, B. Enkhjargal and D. Banzragch, 2019. Morphological and molecular identification of *Beauveria bassiana* from agricultural soils. Mong.J.Agric.Sci. **27** (02): 20-24.

Sabbour, M.M., M. Ragei and A. Rahman, 2011. Effect of some ecological factors on the growth of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against corn borers. Australian J. Basic and Appl. Sci. **5**(11) : 228-235.

Sookar, P., S. Bhagwant and O.E. Awuor, 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae).J. Appl. Entomol. **132**: 778–788.

Thakur, R. and S.S. Sandhu, 2010. Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of central India J. Microbiol.**50**: 89-96.

Verma, P., V.K. Nirmalkar, H. Rajwade, and R.K.S.Tiwari, 2023. Field efficacy of *Lecanicilliumlecanii* and combination of entomopathogenic fungi against rice stem borer (*Scirpophaga incertulas* L.) and leaf folder (*Cnaphalocrocis medinalis* L.) under natural field condition, J. Soils and Crops. **33**(1): 99-105.

Vuillemin, P. 1912. *Beauveria* nouveau genre de verticilliacies. Bulletin de la Social botanique de France. **59**: 34-40.

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