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CULTURAL AND MORPHOLOGICAL VARIABILITY AMONG THE ISOLATES OF *RHIZOCTONIA BATATICOLA* (TAUB.) BUTLER, CAUSING CHICKPEA DRY ROOT ROT

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ABSTRACT

Twelve chickpea isolates of *Rhizoctonia bataticola* were obtained from different Marathwada region of Maharashtra state in 2021–22 and 2022-23. The isolates were examined for their morphology and cultural characteristics. The colony color of 5 isolates was blackish gray, 4 isolates was grayish white, two isolates was with charcoal black pigmentation and rest of only one was light black. Three isolates had appressed colony texture, while 5 isolates had velvety and 4 isolates had fluffy. Ten isolates were categorized as fast growing, 2 isolates were categorized as medium growing and no any isolate was categorized as slow growing. The mean sclerotial sizes of the isolates varied between 48.50 to 126.00 µm. The sclerotia of the 5 isolates were irregular, 4 isolates were round and 3 isolates were ovoid shaped. The isolates were highly variable in their morphological and cultural characteristics.

Key words : *Rhizoctonia bataticola*, Chickpea, Symptoms, Dry root rot, Pathogenicity, Hyphal tip.

Introduction

Chickpea (*Cicer arietinum* L.) is the only cultivated species in the genus *Cicer* and is a self-pollinated diploid (2n=2x=16) crop. It is a cool season legume crop grown worldwide as a food crop. The seed is the main edible part of the plant. It is also called garbanzo gram or bengal gram. Chickpea is a *rabi* crop preferably sown in September-November and harvested in February. Crop matures in around 90-120 days, depending on variety. Areas having low to moderate rainfall and mid-cold weather are best suitable for the crop, preferably moderate rainfall of 60-90 cm per annum. It has indeterminate growth habit, which means that the growth cycle extends as long as moisture is available.

India is a major chickpea producing country during 2021-22 and the crop was cultivated in an area of 98.96 lakh ha with an annual production of 107.37 lakh tonnes with the productivity of 1086 kg/ha whereas in Maharashtra occupies an area of 19.80 lakh ha with total production of 19.17 lakh tonnes, respectively with productivity of 968 kg/ha (FW (DA&FW), Govt of India; Annual Report, 2021-22), while in Marathwada region it was cultivated in an area of 22.31 lakh ha with production and productivity of 23.96 lakh tonnes and 1192 kg/ha, respectively (Anonymous, 2022). Fifty-five pathogens have been so far reported on chickpea right from early seedling stage up to maturity and several of them are of economic importance. Among the several soil-borne fungal diseases, dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is the most severe disease of chickpea especially in the central and southern zones, where the crop is mostly grown in *rabi* season under rainfed conditions.

Predominantly, disease appears around flowering and podding stage. The first symptom is yellowing and sudden drying of the plants. The tap root becomes dark brown quite brittle in dry soil and shows extensive rotting resulting in the loss of lateral roots. The lower portion of the tap

root is often left in the soil when plant is uprooted. Dry root rot caused 10-25 per cent crop losses in major chickpea growing states of India (Lakhran and Ahir, 2018). Also in Marathwada region of Maharashtra state with an average 16.12 per cent incidence was found (Kadam *et al*., 2018). Therefore, the objective of this research to undertaken the study of morphological, cultural and identification of *R. bataticola* isolates causing dry root rot obtained from chickpea in Marathwada region of the Maharashtra State.

Materials and Methodology

Variability among *R. bataticola* isolates

Cultural variability

Twelve test isolates of *R. bataticola* (Taub.) Butler, were aseptically inoculated separately on autoclaved and cooled PDA plates and incubated at 28 ± 2^{0}C. For each test isolate, a triplicate set of PDA plates were maintained. Observations on cultural characteristics *viz*., colony diameter, colony colour, growth rate, colony texture, colony colour (reverse) and margin were recorded, at seven days of incubation.

Morphological variability

For morphological characteristics, temporary mount of a weak old pure culture of the test isolates were prepared separately in a drop of Lactophenol cotton blue stain, on the clean glass slide, covered with glass cover slip and mounted under research microscope. The hyphal width (µm) was recorded, by employing micrometry under five random microscopic fields (40X) for each test isolate and calculated their average hyphal width (Aneja, 2001). From the pure culture PDA plates (two weeks old) of the test isolates, observations on microsclerotia characters *viz*., shape, colour, size (µm) and number / microscope filed (Stereobinocular microscope) were recorded. Observations on number of microsclerotia produced by the test isolates were recorded under five random fields of stereobinocular microscope and averaged the data. The test isolates were categorized on the basis of microsclerotia production (density) / microscopic field, as detailed below (Das, 1988).

S. no.	Grade	Description	Av. no. of sclerotia /microscopic field
1.	-	No sclerotial formation	-
2.	+	Poor sclerotial formation	10-20
3.	+ +	Fair sclerotial formation	21-30
4.	+ + +	Good sclerotial formation	31-50
5.	+ + ++	Excellent sclerotial formation	>51

Results and Discussion

Cultural variability

Results (Table 1, Plate 1 and Fig. 1) showed that all of the 12 test isolates of *R. bataticola*, expressed significant differences in respect of cultural characteristics on PDA medium after seven days of incubation. The colony diameter (mm) of the test isolates were ranged from 79.50 to 90.00 mm and the growth rate was slow, medium and fast.

Based upon the colony diameter and growth rate the test isolates were grouped as fast, medium and slow growing. The fast-growing isolates with colony diameter of 81.00-90.00 mm were Rb-1, Rb-2, Rb-3, Rb-4, Rb-5, Rb-7, Rb-8, Rb-9, Rb-10 and Rb-11; medium growing isolates with colony diameter of 71.00- 80.00 mm were Rb-6 and Rb-12 and none of the isolate belonged to the category of slow growing. The colony colour varied from light black to blakish gray, blackish grey colonies were recorded in five isolates (Rb-2, Rb-4, Rb-6, Rb-10 and Rb-12), whereas charcoal black pigmentation was recorded in two isolates (Rb-1 and Rb-11), while grayish white colony colour exhibited by (Rb-3, Rb-5, Rb-7 and Rb-9) and light black colour of colony was noticed in only one isolate (Rb-8).

The colony texture of the test isolates were categorized in three groups such as appressed, velvety and fluffy. Out of twelve isolates, isolates (Rb-1, Rb-6 and Rb-10) showed appressed colony texture while isolates (Rb-2, Rb-4, Rb-8 and Rb-12) had exhibited fluffy texture. Whereas, remaining isolates (Rb-3, Rb-5, Rb-7, Rb-9 and Rb-11) produced velvety type of texture.

The colony elevation was found to be raised in the seven isolates (Rb-1, Rb-2, Rb-4, Rb-6, Rb-8, Rb-10 and Rb-12); whereas, it was noticed flat in the five isolates *viz*., Rb-3, Rb5, Rb-7, Rb-9 and Rb-11. The colony margin was recorded wavy in the five isolates (Rb-1, Rb-4, Rb-6, Rb-9 and Rb-11); whereas, it was smooth in the seven

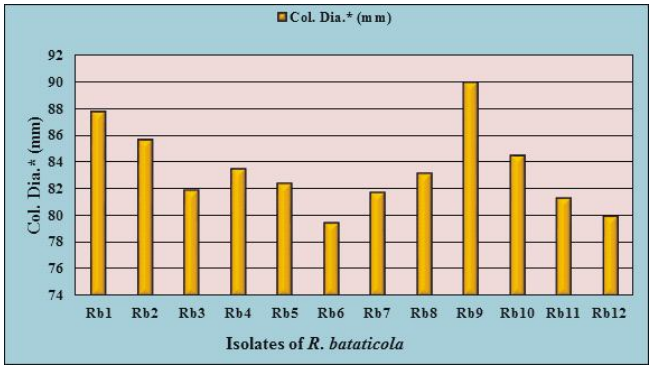

Fig. 1 : Cultural variations among the different isolates of *R. bataticola*.

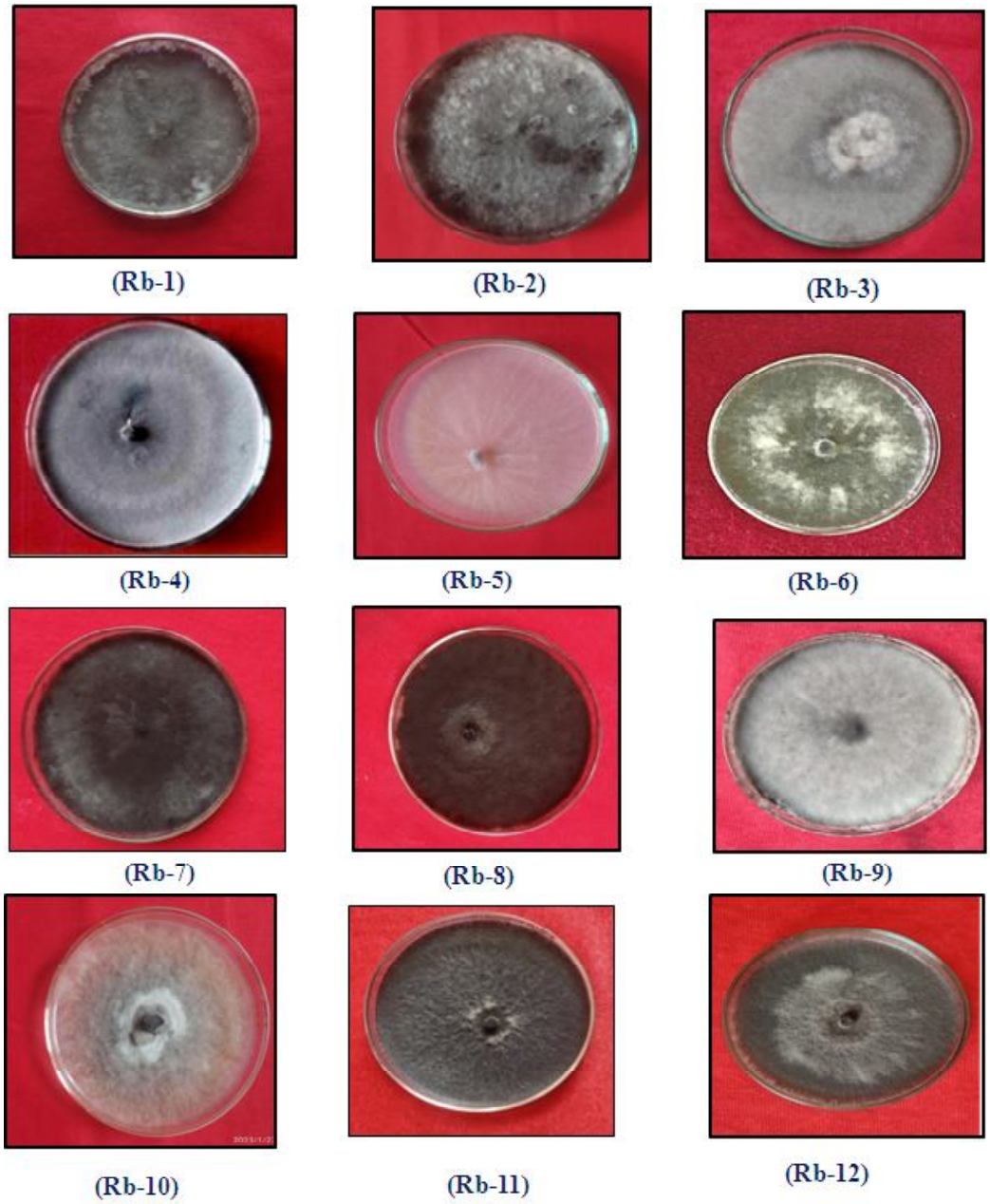

Plate 1 : Cultural variability among the test isolates of *R. bataticola.*

isolates *viz*., Rb-2, Rb-3, Rb-5, Rb- 7, Rb-8, Rb-10 and Rb-12).

Morphological variability

The results on morphological characteristics such as hyphal width and sclerotial characters like shape, colour and size with respect to the test isolates of *R. bataticola* are presented in (Table 2 and Plates 2 & 3).

Average hyphal width of the test isolates was ranged from 3.19 to 7.90 µm. Based on average hyphal width, the test isolates were classified into three categories *viz*., large (>5.00 µm), medium (4.00-4.99 µm) and small (<4.00 µm). Among the 12 isolates, seven isolates were found with large sized hyphae (5.05-7.90 µm) *viz*., Rb-1, Rb-2, Rb-4, Rb-6, Rb-9, Rb-10 and Rb-11; four isolates were found with medium sized hyphae *viz*., Rb-3, Rb-5, Rb-7 and Rb-12 and one isolate was found with small sized hyphae *viz*., Rb-8.

The color of sclerotia was brown to black and their shape was observed round, ovoid to irregular one. Most of the isolates produced *viz*., Rb-3, Rb-5, Rb-9, Rb-10 and Rb-12 irregular shaped sclerotia and rest three isolates *viz*., Rb-1, Rb-6 and Rb-8 were ovoid shaped and four isolates *viz*., Rb-2, Rb-4, Rb-7 and Rb-11 exhibited round shaped sclerotia.

All the test isolates showed considerable variations in respect to no. of sclerotia produced (density /

Table 1 : Cultural characteristics of twelve isolates of *R. bataticola* on PDA medium.

S. no.	Isolates	Col. Dia.* (mm)	Growth rate	Colour	Colony texture	Elevation	Margin
1.	Rb_1	87.80	(++++)	Charcoal black	Appressed	Raised	Wavy
2.	Rb_2	85.75	(++++)	Blackish Grey	Fluffy	Raised	Smooth
3.	Rb_3	81.90	(++++)	Grayish White	Velvety	Flat	Smooth
4.	Rb_4	83.50	(++++)	Blackish Grey	Fluffy	Raised	Wavy
5.	Rb_5	82.45	(++++)	Grayish White	Velvety	Flat	Smooth
6.	Rb_6	79.50	(+++)	Blackish Grey	Appressed	Raised	Wavy
7.	Rb_7	81.75	(++++)	Grayish White	Velvety	Flat	Smooth
8.	Rb_8	83.20	(++++)	Light black	Fluffy	Raised	Smooth
9.	Rb_9	90.00	(++++)	Grayish White	Velvety	Flat	Wavy
10.	Rb_{10}	84.58	(++++)	Blackish Grey	Appressed	Raised	Smooth
11.	Rb_{11}	81.30	(++++)	Charcoal black	Velvety	Flat	Wavy
12.	Rb_{12}	80.00	(+++)	Blackish Grey	Fluffy	Raised	Smooth

*Average of a triplicate set of PDA plates per isolates, Growth Rate = Fast: ++++ (81.00- 90.00 mm), Medium: +++ (71.00-80.00 mm), Slow: ++(<70.00 mm).

Table 2 : Hyphal width, colour, size and shape of sclerotia among the twelve isolates of *R. bataticola* grown on PDA medium.

S. no.	Isolates	Av. Hyphal width* (µm)	Sclerotial characters		
			Av. Size* (µm) Range	Density# / Microscopic field	Shape
1.	Rb_1	6.90	118.00	64.50	Ovoid
2.	Rb_2	6.35	105.50	60.00	Round
3.	Rb_3	4.72	70.50	31.76	irregular
4.	Rb_4	5.88	90.00	49.32	Round
5.	Rb_5	4.93	76.50	37.87	irregular
6.	Rb_6	5.05	80.00	41.36	Ovoid
7.	Rb_7	4.60	62.50	29.47	Round
8.	Rb_8	3.19	48.50	15.63	Ovoid
9.	Rb_9	7.90	126.00	72.00	irregular
10.	Rb_{10}	5.47	83.50	45.66	irregular
11.	Rb_{11}	6.10	94.00	53.68	Round
12.	Rb_{12}	4.37	55.00	20.86	irregular

*: Mean of ten observations / isolates, #: Mean of five microscopic fields (40X).

microscopic field) and their size. Sclerotial production among the test isolates were grouped as poor (10-20 sclerotia / microscopic field), fair (21-30 sclerotia / microscopic field), good (31-50 sclerotia / microscopic field) and excellent (>51 sclerotia / microscopic field). All-inclusive, sclerotial density was ranged from 15.63 to 72.00. However, the sclerotial density was found excellent in four isolates such as Rb-9 (72.00), Rb-1 (64.50), Rb-2 (60.00) and Rb-11(53.68); good in five isolates *viz.*, Rb-3 (31.76), Rb-4 (49.32), Rb-5 (37.87), Rb-6 (41.36) and Rb-10(45.66); fair in only one isolate *viz.*, Rb-7 (29.47) and poor in two isolates *viz.*, Rb-8 (15.63) and Rb-12 (20.86).

Average size produced by the sclerotia of the test isolates was ranged from 48.50 to 126.00 µm. Depending upon the size of the sclerotia, the test isolates were Classified as large sized sclerotia (>101 µm), medium (50- 100 µm) and small (<50 µm). The large sized sclerotia were observed in three isolates *viz.*, Rb-9 (126.00 µm), Rb-1 (118.00 µm) and Rb- 2 (105.50 µm); medium sized sclerotia were observed in six isolates *viz.*, Rb-12 (55.00 µm), Rb-7 (62.50 µm), Rb-3 (70.50 µm), Rb-5 (76.50 µm), Rb-6 (80.00 µm), Rb-10 (83.50 µm), Rb-4 (90.00 µm) and Rb-11(94.00 µm) and small sized sclerotia was found in only one isolate *viz.*, Rb-8 (48.50 ìm).

Present results obtained on morpho-cultural variability among the isolates of *R. bataticola* causing dry root rot of chickpea are in consensus with those findings of several other workers *viz.*, Aghakhani and Dubey (2009a) reported morpho-cultural variability among 23 isolates of

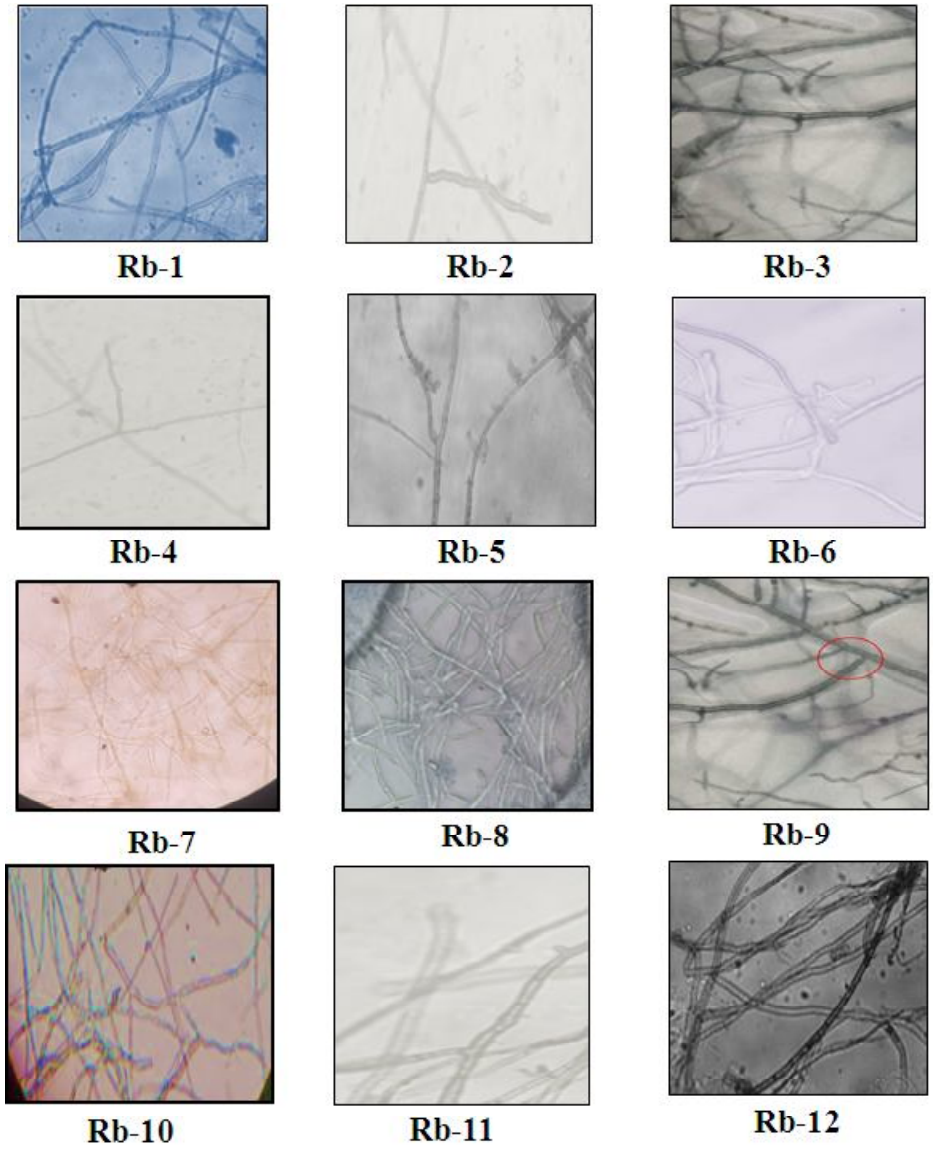

Plate 2 : Morphological variability among the test isolates of *R. bataticola*.

R. bataticola. All test isolates were also found to vary in respect of morpho-cultural characteristics. Sharma *et al*. (2012a, b) also studied morpho-cultural variability among 94 isolates of *R. bataticola* causing dry root rot of chickpea and reported all test isolates varied in morpho-cultural characteristics. Sharma *et al*. (2015) also reported that *R. bataticola* causing dry root rot of Chickpea grew better on PDA and produced brown to gray coloured mycelium, which turned darker with age. The young hyphae were thin, hyline, aseptate and dichotomously branched, latter produced black sclerotia. The sclerotia formed were black, smooth, spherical or oblong to irregular shaped. Similarly, Gade *et al*. (2018) studied morphological variability and 40 isolates of *R. bataticola* causing dry root rot of soybean. Maximum sclerotial size was reported in isolate Rb-33 (120.11 µm) while, the isolate Rb-40 produced smallest size sclerotia (42.03 µm). Average sclerotial size of the isolates ranged from 42.03-120.11 µm. Large sized sclerotia (>80 µm) were produced by 16 isolates, eight isolates had medium sized sclerotia (60-80 µm) and rest 16 isolates had small sized sclerotia (<60 µm). Basbagcia and Dolar (2022) also studied morpho-cultural variability among 19 isolates of *R. bataticola*, causing chickpea dry root rot. They reported that colony color of 10 isolates were grayish black and rest of them were black. Eleven isolates had appressed colony texture while three isolates had velvety and five isolates had fluffy. Eight isolates produced aerial mycelium and eight isolates were categorized as fast growing, eight isolates were categorized as medium growing and 3 isolates were categorized as slow growing. The sclerotia of the 11 isolates were round 3 isolates were ovoid and 5 isolates were irregular shaped. Sunkad *et al*. (2023) studied morpho-cultural variability among 60 isolates of *R.*

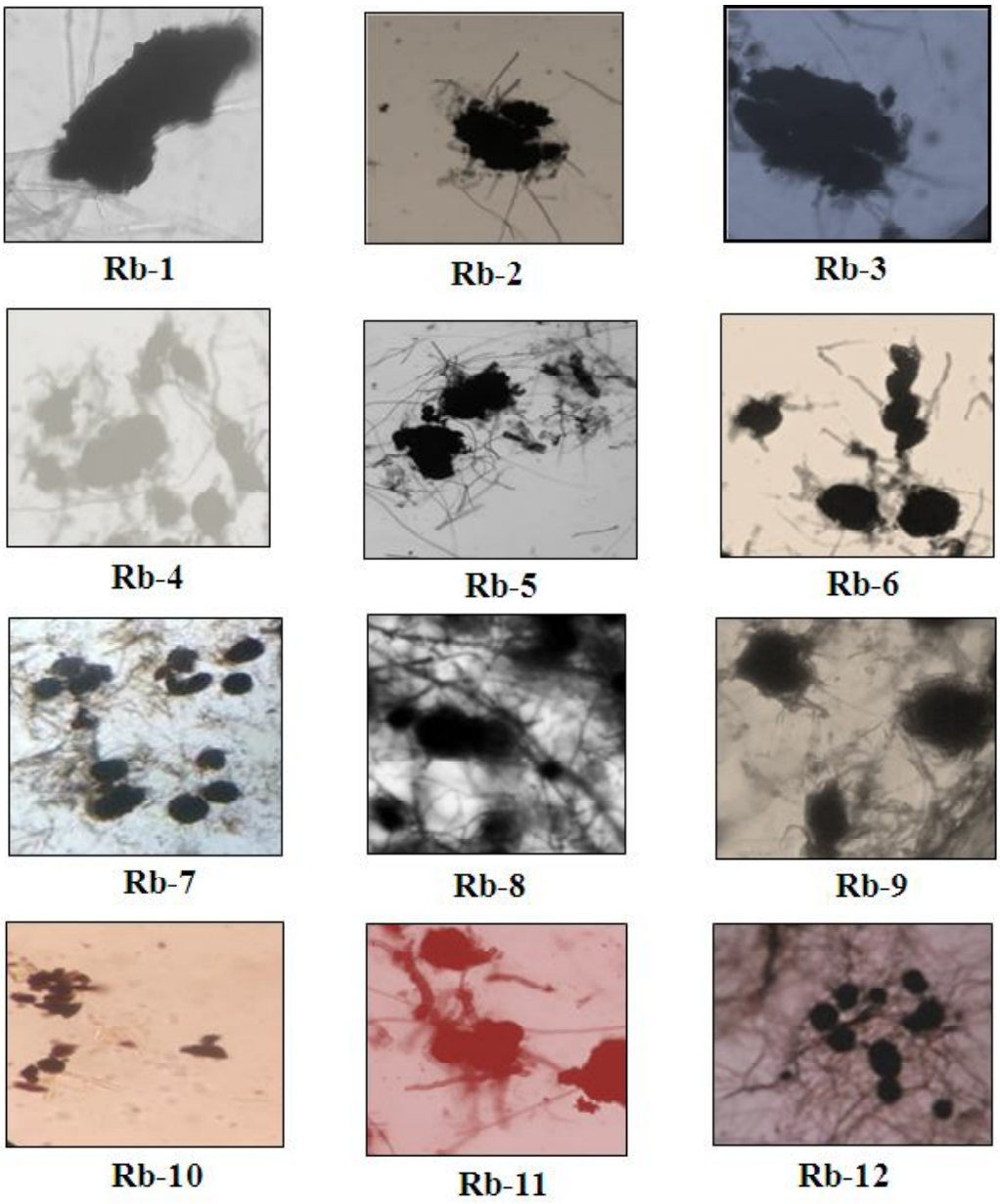

Plate 3 : Morphological variability among the test isolates of *R. bataticola*.

bataticola, causing chickpea dry root rot. They reported that radial growth of *R. bataticola* isolates ranged from 23.90-90 mm. Fifty-five isolates were fast growing (75-90 mm), colony morphology of 17 isolates recorded appressed growth, 13 velvety and rest of the 30 isolates showed fluffy growth. Aerial mycelium of 28 isolates showed the production of aerial mycelia and in 32 isolates aerial mycelia was absent. Fifty-one isolates produced microsclerotia, while 9 did not produce microsclerotia. Twenty-one isolates produced sclerotial bodies in the range of 1-25 per microscopic field (10x), 26 (26-50 range) and four isolates (51-100 range). Round shaped microsclerotia were observed in twenty isolates, ovoid (13) and irregular (18).

Conclusion

There was more diversity in *R. bataticola* isolates with respect to cultural and morphological characters. The determination of variability among *R. bataticola* isolates is fundamental to guide the development of appropriate strategies for disease management according to different agro ecological zones. The present studies provide information on the morpho-cultural variability of *R. bataticola* in major chickpea growing Marathwada regions of Maharashtra. These results will be useful in developing integrated strategies for the management of chickpea dry root rot and breeding programs for pulses and other crops.

Author contribution

S.S. Kadam recorded and analysed observations. S.S. Kadam and S.N. Banne, wrote the manuscript. D.G. Hingole, conceived and designed the research and guided during period of study.

Conflict of interest

There is no conflict of interest regarding the manuscript among the authors.

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