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In vitro studies on susceptible reactions of groundnut varieties to *Macrophomina* infection in relation to varied seed coat colour

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Abstract
Seed health is an important factor in the control of diseases, as an infected seed exhibits low germination, viability, vigour and yield. Among seed borne mycoflora of groundnut, an infection caused by *Macrophomina phaseolina*, called dry root rot (DRR) is most devastating due to its polyphagous, seed and soil-borne nature. Managing it with the use of resistant varieties is one of the economically congenial methods. The present study aimed to identify the resistant varieties based on their seed coat colour against *Macrophomina* infection by using the paper towel method. Out of fifteen varieties, we identified 4 varieties as resistant to infection without any seed mortality, seven varieties were moderately resistant and another 4 varieties were found to be susceptible with 20 percent seed mortality in the repeated experiments. Results revealed a significant reduction in seedling vigour index in red-coloured cultivar *i.e.*, Local variety (89.34%). The lowest reduction in seedling vigour index was found in salmon-coloured cultivar *i.e.*, SB-XI (14.24%).

Keywords: Groundnut, seed borne mycoflora, seed coat colour, seed vigour

1. Introduction
Groundnut (*Arachis hypogaea* L.) is one of the most important and extensively cultivated oil seeds (4th most) and staple food legume crop (13th most) from the leguminaceae family (Surendranatha *et al*., 2011) [14]. Groundnut is an important crop cultivated around the world and is grown in 26.89 Mha with a remarkable total production of 46.06 million metric tonnes and productivity of 1.71 million metric tonnes per hectare, with developing countries in Asia (66%) and Africa (25%) as the major producers (USDA-FAS 2019-20) [15]. Worldwide largest top five groundnut-producing countries are China, India, Nigeria, the United States and Sudan with an average production of 17.52, 6.26, 3.50, 2.46 and 1.80 million metric tonnes respectively (USDA-FAS 2019-20) [15]. In India, it is mostly grown in the states like Gujarat, Andhra Pradesh, Rajasthan and Maharashtra. Maharashtra occupies sixth place under the cultivated area with 244.12 thousand hectares and stands in eighth place in production (MOAFW, GOI (ON2331), 2018-19).
Out of nine oilseed crops, it is regarded as the "King of Oilseeds and Poor Man's Cashewnut" as it contains a good source of protein, oil and fibres (Vijay Kumar, 2007) [17]. Oil extracted from groundnut seeds is mostly used in cooking. The seeds are edible and their rich proteinaceous nature makes them suitable for the preparation of peanut butter, which has growing demand. It holds many valuable energy sources of oil (48-50%) and protein (25-28%) in the kernel, providing 564 kcal of energy from 100 g of kernels (Jambunathan, 1991; Blummel *et al*., 2005) [7, 4]. Despite the extensive uses of the groundnut and rapid cultivation of the crop, a disheartening trend is that the productivity of the groundnut crop is reduced in the recent past. The full potential of the crop is so far from being exploited, due to the low level of yield in India compared to the global level, which is attributed to several biotic and abiotic factors influencing the seed quality. Pre- and post-harvest pathogen infections are creating a menace to groundnut production due to changing climatic conditions and lack of proper storage facilities at the post-harvest stage. Seed health plays an important role in plant disease control, since an infected seed has less germination, vigour and viability (Van Gastel *et al*., 1996) [16]. Several studies revealed that seeds and germinating seedlings of groundnut contain mycoflora like *Alternaria* sp., *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *Myrothecium roridum*, *Drechslera* sp., *Aspergillus flavus*, *A. niger* and *Macrophomina phaseolina* etc., (Bakr and Rahaman, 2001) [3].

Among these *M. phaseolina* is an important seed pathogen which causes a devastating symptom like pre-emergence seedling rot, mortality and DRR in post emergence conditions in groundnut.

It is known that the first line of the defence mechanism in plants involves seed coat, the most important structural and biochemical barrier to invading pre-and post-harvest pathogen infections. In general, when we remove seed coat all the varieties of groundnut exhibit the same levels of fungal infection (Yasseen *et al*., 1994) [20]. Presently, the information on the effect of *M. phaseolina* on groundnut seed health parameters like seed germination and SVI is inadequate in relation to their seed coat colour. Keeping this in view, the present investigation was envisaged to find the resistant sources from different seed coat-coloured varieties.

2. Materials and Methods

This investigation was carried out to study the effect of *M. phaseolina* on different seed coat-coloured groundnut varieties by analyzing the parameters like seed germination and SVI in the Department of Plant Pathology, MPKV, Rahuri. Fifteen varieties of groundnut were collected from markets of Andhra Pradesh, Maharashtra and AICRP unit, Rahuri. The varieties were grouped based on seed coat colour variation *viz.,* red, pink and salmon colours as described in Satpute and Santosh. 2011 [13]; Nayak *et al*., 2020 [10]; Chukwumah *et al*., 2009 [5] and presented in Table 1.

2.1 Preparation of *Macrophomina* spore suspension

Seed-borne *Macrophomina phaseolina* was obtained from the collected groundnut seeds with the standard agar plate method as per ISTA (1985) [6] shown in Figure 1. Pure culture of the pathogen was obtained with hyphal tip method shown in Figure 2.

Spore suspension were separately prepared for each variety from pure culture disc of sporulating fungi by adding in 100 ml of autoclaved Potato Dextrose Broth (PDB) aseptically in laminar air flow chamber (Agostini and Timmer, 1992) [2]. Then flasks were incubated for ten days at room temperature. After incubation period the mycelial mat from the flask was removed and macerated with distilled water in a warring blender for few minutes. Then the inoculum was collected in a beaker.

2.2 Effect of *M. phaseolina* on seed health

A total of fifteen different seed coat-coloured groundnut varieties were used to study the influence of *M. phaseolina* on seed health parameters like seed germination and SVI through the paper towel method (Warham *et al*., 1990; Rameela *et al*., 2018) [19, 12]. Four hundred seeds from each variety as per their seed coat colour were first sterilized with 1% sodium hypochloride solution and washed three times with distilled water, then completely immersed in spore suspension overnight.

The next day, one sheet of paper towel or germination paper (45 cm × 25 cm) was wetted with distilled water. Later overnight inoculated seeds (25) of respective groundnut cultivars were blot dried and placed on the first sheet side by side evenly (5 in each row) and the wetted second sheet of germination paper was carefully placed on the first sheet. Again, both sheets were wetted and rolled along with wax-coated paper to prevent drying, tied at both ends with proper labelling. Then these were placed in trays and incubated in a seed germinator at 30 °C for 10days. Simultaneously, sterilized seeds without inoculation were kept as a control. Four replications each of 100 seeds were maintained for each treatment. Every day the paper towels were moistened with sterile distilled water. At the end of incubation, rolled germination papers were carefully opened and noted down the number of germinated and ungerminated seeds with respect to variety and colour. Then these seeds were used for further study to record seedling abnormalities, seed germination and SVI.

2.3 Seedling vigour index (SVI)

It was calculated on the basis of seed germination and seedling length (mean root length and shoot length) after challenging seeds with *M. phaseolina* for seven days of incubation at 25 °C for 7 days in the paper towel method (Abdul-Baki and Anderson, 1973) [1].

Seedling Vigour Index (SVI) = (Mean root length + Mean shoot length) × Seed germination (%)

2.4 Statistical analysis

The data of *Macrophomina* infection on seed germination, percent mortality and SVI were translated into Arc sin transformation and then statistical analysis was performed as suggested by Panse and Sukhatme (1985) [21]. The average percent seed germination, mortality and decrease in SVI over control was translated into Arc sin transformation.

3. Results and Discussions

3.1 *In vitro* studies on the effect of *M. phaseolina* on seed germination & SVI of different groundnut varieties with respect to seed coat colour

Seed germination and its, SVI was greatly affected by seed mycoflora. The obtained results of the effect of *M. phaseolina* (Artificially inoculated to seeds through paper towel method) on seed germination and SVI as per their seed coat colour are shown in Table 2 and Figures 3 & 4. It was evident that red-coloured varieties *i.e.,* Local variety-1, 2 showed susceptible reactions, while pink-coloured varieties *i.e.,* KDG-160, Phule-Unnati showed intermediate reactions, whereas Salmon-coloured varieties *i.e.,* SB-XI, JL-1085 showed resistant reactions.

In general varieties recorded an increase in percent mortality, a decrease in the seed germination and seedling vigour index over control for *Macrophomina* infection (Table 2). The percent mortality of groundnut seedlings to *M. phaseolina* inoculation was recorded highest in red-coloured varieties such as Local variety-1 and 2 (20.00%) over control followed by TPG-41 (16.04%). Whereas, seedling mortality was not noticed in salmon-coloured varieties namely SB-XI and JL-1085(no mortality) over control. The decrease in seed germination and seedling vigour index to *M. phaseolina* inoculation was noticed highest in red-coloured varieties namely Local variety-1 with 52.00 and 89.34 percent, respectively. While a decrease in seedling vigour index was noted lowest *i.e.,* 14.24 and 16.10 percent, respectively for salmon-coloured varieties such as SB-XI and JL-1085 over control.

Table 1: Grouping of collected groundnut varieties based on the intensity of seed coat colour

S. No.	Variety Name	Variety	Colour & Hex code
1.	Local variety-1		Dark-Red (#842727)
2.	TPG-41		Red-Brown-1 (#682do9)
3.	Local variety-2		Red-Brown-2 (#7a2b0b)
4.	RHRG-1192		Red-Brown-3 (#77340b)
5.	KDG-160		Pink-1 (#f98d75)
6.	Phule-Unnati (RHRG-6083)		Pink-2 (#f88066)
7.	Phule-Warna		Pink- 3 (#fa9a85)
8.	JL-776		Pink-4 (#faao8d)
9.	RHRG-1308		Pink-5 (#faa795)

10.	Phule-Pragathi (JL-24)		Pink-Peach-1 (#fbbfb5)
11.	TAG-24		Pink-Peach-2 (#fbc6bc)
12.	JL-501		Salmon-1 (#e59879)
13.	Phule-6021		Salmon-2 (#e06c45)
14.	JL-1085		Salmon-3 (#ffa07b)
15.	SB-XI		Salmon-4 (#ffb294)

Fig 1: Isolation of Seed borne *M. phaseolina* from collected groundnut seeds by standard agar plate method

Fig 2: Pure culture of *M. phaseolina* (left) and sclerotia (right)

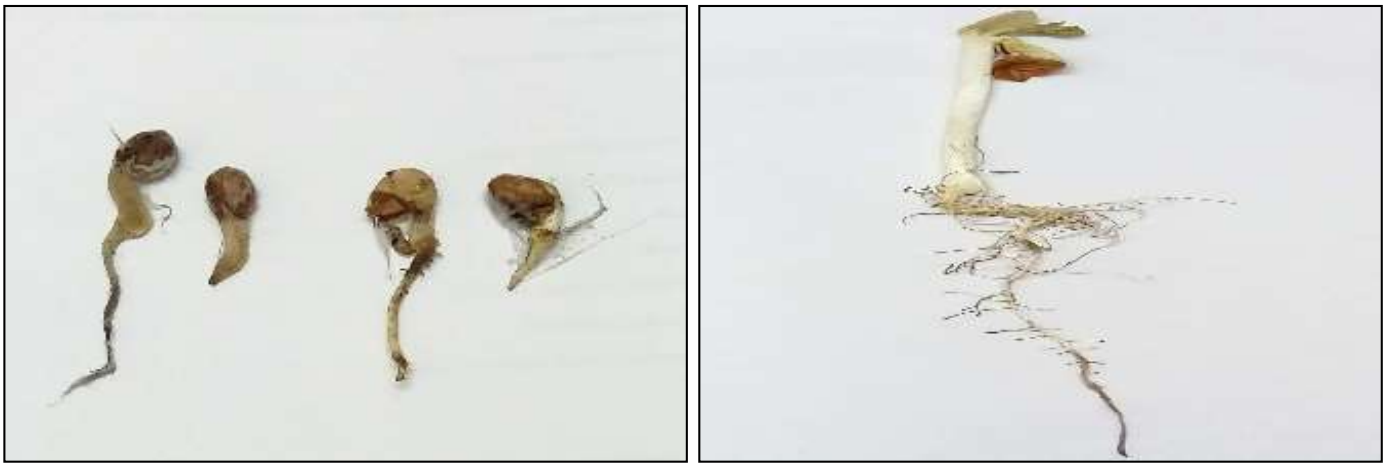

Fig 3: Seedling rot symptoms of *M. phaseolina* (left) and a healthy seedling (right)

Fig 4: Resistance/susceptible reactions of *Macrophomina* on a) Local variety 1 (Dark Red; top left)), b) KDG-160 (Pink; top right)) and c) SB-XI (Salmon colour; bottom))

Table 2: *In vitro* studies on the effect of *M. phaseolina* on seed germination and seedling vigour index of groundnut

Sl. No.	Variety	Seed coat colour	Seed Germination (%)	Percent mortality		Mean	SVI	Reduction in seed germination over control (%)	Reduction in SVI over control (%)
				Pre Emergence	Post Emergence				
1.	Local variety-1	Dark Red	32.00 (34.45)	20.00 (26.57)	20.00 (26.57)	20.00 (26.57)	102.00	52.00 (46.15)	89.34 (70.95)
2.	TPG-41	Red Brown-1	40.00 (39.23)	15.92 (23.51)	16.17 (23.71)	16.04 (23.61)	482.57	48.00 (43.85)	62.69 (52.35)
3.	Local variety-2	Red Brown-2	36.00 (36.87)	20.00 (26.57)	20.00 (26.57)	20.00 (26.57)	92.02 (73.59)	44.00 (41.55)	87.97 (75.80)
4.	RHRG-1192	Red Brown-3	80.20 (63.55)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	836.20	3.8.00 (11.24)	59.55 (50.51)
5.	KDG-160	Pink-1	68.00 (55.55)	16.00 (23.58)	16.00 (23.58)	16.00 (23.58)	543.21	20.00 (26.57)	61.28 (51.52)
6.	Phule-Unnati	Pink-2	92.00 (73.57)	14.15 (22.10)	0.00 (0.00)	7.08 (15.43)	1334.45	8.00 (16.43)	28.99 (32.58)
7.	Phule-Warna	Pink-3	96.00 (78.46)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)	1550.80	4.00 (11.54)	26.39 (30.92)
8.	JL-776	Pink-4	84.00 (66.42)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1102.23	16.00 (23.58)	49.55 (44.74)
9.	RHRG-1308	Pink-5	76.20 (60.78)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	638.69	19.80 (26.42)	55.17 (47.97)
10.	JL-24	PinkPeach-1	96.20 (78.71)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1826.67	3.80 (11.24)	21.53 (27.65)
11.	TAG-24	PinkPeach-2	84.00 (66)	12.00 (20.27)	12.17 (20.41)	12.08 (20.34)	733.18	16.00 (23.58)	67.28 (55.11)
12.	JL-501	Salmon-1	92.70 (74.29)	8.17 (16.61)	0.00 (0.00)	4.08 (11.66)	1844.27	7.30 (15.68)	23.90 (29.27)
13.	Phule-6021	Salmon-2	96.20 (78.71)	16.00 (23.58)	8.00 (16.43)	12.00 (20.27)	882.82	3.80 (11.24)	25.29 (30.20)
14.	JL-1085	Salmon-3	96.00 (78.46)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1886.68	4.00 (11.54)	16.10 (23.66)
15.	SB-XI	Salmon-4	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1344.31	0.00 (0.00)	14.24 (22.17)
	SEM		0.19	0.04	0.06		0.50		
	CD @ 1%		0.60	0.13	0.17		1.51		

*Figures in parentheses indicate arc sin transformed values

4. Conclusion and future perspective

From this study, out of fifteen groundnut varieties examined for their reaction against *Macrophomina* with the paper towel method, four salmon-coloured genotypes were found resistant. Seven pink-coloured genotypes were moderately resistant, while, four red-coloured varieties were susceptible. Thus, the selected genotypes containing the above-selected characters can be considered for a further selection of resistant lines under field trails which will be useful for the development of resistant varieties in breeding programmes against DRR resistance. The studies on the seed coat colour of groundnut for their reaction to *Macrophomina* infection concluded that utilization of genotypes showing resistance reactions to *Macrophomina* is the better alternative control measures in disease-prone areas since it is environmentally and economically safe and can be easily used in IDM (integrated disease management) in combination with other control measures.

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