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Exploring Host Plant Resistance of Cowpea as for *Macrophomina Phaseolina* (Tassi.) Goid) Causing Dry Root Rot Pathogen under Artificial Inoculation Condition

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Abstract

Background: Cowpea (*Macrophomina phaseolina* (Tassi.) Goid) is accepted as a very critical commercial crop belong to arid and semi-arid region. Among various diseases of cowpea dry root rot caused by M.P. is a major constrain to drop down the significant yield loss as factor that it was serve as both seed and soil borne with wide host range. Dry root rot disease in cowpea causes the lowering of average yield as compared to its potential yield. The current study greatly emphasized to find out suitable resistance genotype against cowpea and adverse remark of dry root rot on yield

Methods: Screening was conducted against nineteen cowpea genotypes to find out the resistance reaction against dry root rot under artificial inoculated condition between for three consecutive three year that is 2019, 2020, and 2021.

Conclusion: Cowpea genotypes were explored to find out the resistant source against dry root rot of cowpea, none of the entry was found free from dry root rot. The data on severity index reveals that the highest disease severity index was recorded with accession GC-1712 (91.69 %), followed by KBC-11 (88.91 %), TC-1901 (80.53%), CPD-311 (75.00 %), KBC-13 (72.22 %), DC-15 (63.90 %), PCP 1124-1 (20.54 %), PCP-0306 (19.99 %), KBC-9 (6.67%), RC-101 (5.56 %), CPD-313 and GC-1602 (5.00 %), GC-3 (4.44 %), Pant lobia-3, PGCP-69 and TPTC-29 (3.89 %), PGCP-70 (2.22 %), while disease severity index minimum was recorded in accession Pant lobia-4 and MC-17-2 (KBC-12) (1.11 %) respectively. Eleven entries *viz.*, GC-3, TPTC-29, RC 10, KBC-9, Pant Lobia-3, Pant Lobia-4, MC 17-2 (KBC-12), CPD-313, PGCP-69, GC-1602, PGCP-70 were found moderate resistant, two entries PCP-0306 and PCP 1124-1 were moderate susceptible and six entries DC-15, CPD-311, KBC-13, KBC-11, TC-1901 and GC-1712 were highly susceptible.

Key word: Screening, cowpea, genotypes, root rot.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) was considered as protein source for poor men's and be always a critical component for humans diet as a source of protein. From the ancient ages cowpea is considered as a food sources for human beings (1985)[1] and also it served grain legume as well as hay crop in many of tropical and subtropical countries (2007)[2]. About 75% of the global production is acquire from Africa (2002)[3] as well as second most critical pulse crop scrutinized among the best five influential pulse in the world (2013)[4]. Among various pulses, cowpea is cultivated in Andhra Pradesh and Tamil Nadu as rainfed crop. Several terminology has been placed for the crop as "Cowpea" as it is because it serve as cattle feed especially for cowpea also "hungry season crop" as it the first crop to be harvested as another cereals crop (2004)[5]. Beside all the parameters it is also served as source for vegetable, snacks, food, forage and fodder (2001)[6]. It has a freat affinity to fix the atmospheric nitrogen win the soil in association with symbiotic bacteria which are present in its root nodule with a rate of 56 kg per ha (2005, 2000)[7] . Cowpea is a crop grown very suitably in deficit soils i.e., soils with 0.2 percentage of biological matter and more an 85% of sand along with low level of phosphorus in soils. In India it is cultivated in an region of 12.56 lakh hectares particularly in western, central and peninsular regions, with a production of 30.53 lakh tonnes and productivity of about 5.67 kg/ha (Anon 2017)[8]. Globally the cowpea valued on an about 14.5 million ha area each year with annual production of about 6.2 million metric tonnes. Scenario of last three decades justifies that the cowpea average production rate is 5% along with 3.5% annual growth in terms of area and 70% of area is being extensively increased with 15% growth in yield (2016)[9]. In 2020 the cowpea production is goaled to reach to about 9.8 million also the targeted forecasted yield aimed for 2030 is to be 12.3 million tons (2016)[10]. Viruses, fungi and bacteria are the major pathogens leading to cause many diseases in cowpea (2002)[11]. In India the rainfed situation is most preferably suitable for its sowing on about 78% of area under tropical environment which is commending for Cowpea suffered by various diseases incited by various pathogen *viz.*, fungi, viruses and bacteria (Emechebe and Lagoke, 2002)[12]. In India, cowpea and other legume crops are mainly sown under rainfed situations on above 78% of region and being a tropical environment, commending the disease incidence. Fungal pathogen are the top most deadly phytopathogens of cropping plants crops widely (Fisher *et.al.* 2012)[13]. *Macrophomina phaseolina* a fungal pathogen causes charcoal rot causes a very seroius yield losses. The environmental factors heat and moisture stress enhances its cultivation uneconomical (2012.)[14.] One of the fungus originated diseases, the charcoal rot incited by *Macrophomina phaseolina* (Tassi.) Goid causes serious reduction in yield. contemporaneous high temperature and moisture stress favor advancing of charcoal or dry root rot caused by *M. phaseolina* often makes production of cowpea uneconomical (Singh *et al*., 2012)[15]. Incidence of dry root of cowpea ranging from 5 to 39 per cent, (Ushamalini *et al*., 2001)[16]. Various crops *viz.*, oilseed vegetables crops, legumes and huge range of unliked crops were affected by *Macrophomina phaseolina* (2012, 2012, and 2014). Being a seed and soil borne pathogen sometime it encourages the yield losses upto 100% causing a great loss in yield (2014). Old shoot tissue are the primary source for microsclerotia development, they remain alive in soil (2002). Since, the fungus is soil borne which leads to serve problem in controlling dry root rot. Thus the study is especially designed to estimate incidence and severity of dry root rot and most resistance genotypes of cowpea.

2. Method And Materials

Nineteen cowpea genotypes *viz.*, CPD-311, GC-1712, TC-1901, KBC-11, DC-15, KBC-13, PCP 1124-1, PCP-0306, GC-1602, KBC-9, CPD-313, PGCP-69, RC-101, PGCP-70, TPTC-29, GC-3, Pant lobia-3, MC-17-2, and Pant Lobia-4 were screened to find out the resistance reaction against dry root rot under artificial inoculated condition. The healthy and clean seeds were separated from diseased, or damage ones and clean seeds were used for the study. The investigation was carried out in CRBD with replicated twice during the year 2019, 2020 and 2021.

The experimental is conducted at the research farm area at Agriculture college RVSKVV, Gwalior (23^0 10' N latitude, 79^0 54' E longitude) is located in the Central part of Madhya Pradesh at an altitude of 411.98 m from sea level. The experimental field soil collected is sandy with loamy texture and the accessible nitrogen content is very low (237.0 kg/ha), medium range of in phosphorus (19.7 kg/ha) and potassium content is 277.1 kg/ha with pH 7.8. The soil organic carbon was 0.03% and the electrical conductivity of the soil reported 0.25dSm^{-1}. The primary inoculum for the pathogen was isolated from the infected plants and then confirmed in the laboratory.

The inoculum culture was multiplied at the, Department of Plant Pathology laboratory, RVS Krishi Viswa Vidyalaya, Gwalior (M.P.). The soil was previously autoclaved at 121 ^{0}C for 1 hour. Two pots replicate was set-up to nineteen cowpea cultivars. 10 gm of mass multiplied inoculum of pathogen was incorporated around the sown seeds so that it will be easily penetrate to the feeder roots of the germinating seedlings. Five to six healthy seeds of the respective genotypes were placed in each pots. After seven days of sowing the cowpea plants were moisten daily with sterilized distilled water. The observation on disease incidence was taken as percentage in the plants accordance with the symptoms also the presence of first symptoms after the inoculation was also notified. Seedling were also observed for diseases severity and subsequently scored after 7th day of inoculation by using the revised person *et. al.,* 1997 scale as shown below.

- 0= Without any visible symptom categorized as healthy plant
- 5= On root system are discolouration of less than 5 mm.
- 10= On root system are discolouration of about 20 mm.
- 25= Root system are discoloured to about 5%
- 50= But no symptom on the epicotyls or leaves with the whole root system discoloured
- 75= Epicotyls discoloured with the lower leaves wilted also the whole root systems
- 100= Plant dead.

3. Results

Phases involved in the advancement of cowpea root rot disease along with its sign and symptoms in Northern M.P.

The common symptoms were seen during various phases of the dry root rot disease development. A total of nineteen entries of cowpea were evaluated against dry root rot under artificial inoculated condition. The genotypes CPD-311, DC-15 (3 days) were first in which symptoms caused by infection by Macrophomina with artificial insertion method. Chlorosis on leafs and discoloration on lower stems seen appeared as the visible symptoms 3 days after inoculation. The symptoms appeared 4, 5, 6, 7, 8, 12, 15 and days after artificial inoculation for cultivars TC-1901, PCP-1124-1, KBC-13, PCP0306, RC-101, GC-3, GC-1712, KBC-9, TPTC-29, KBC-11, CPD-313, GC-1602, PGCP-69 and PGCP-70, MC-17-2 (KBC-12), Pant Lobia-4 and Pant lobia-3 (Table-1).

Percent incidence and severity of Macrophomina root rot on different genotypes of cowpea.

Prevalence of disease incidence among cowpea genotypes.

The percent incidence for *kharif* 2019 is ranged from 47.50 % to 22.50 %. The genotypes namely KBC-9 (20.00 %), GC-1602 (20.00 %), RC-101, CPD-313, PGCP-69, PGCP-70 (17.50%), GC-3 (15.00 %), TPTC-29 (12.50 %), Pant lobia-3, MC-17-2 (10.00 %), showed moderate resistant reaction, while minimal disease incidence percentage was noticed in genotype Pant Lobia-4 (5.00 %). During *Kharif*-2020 the maximum disease incidence was observed in genotype CPD-311 (49.50 %), followed by GC-1712 (35.50 %), TC-1901 (32.00 %), KBC-11, (30.50 %), DC-15 (28.50 %), KBC-13(28.00 %), PCP 1124-1 (21.50 %), PCP-0306 (21.00 %), GC-1602 (19.50 %), KBC-9 (18.50 %), CPD-313 (17.00 %), PGCP-69,(17.00 %), RC-101(15.50 %), PGCP-70 (15.50 %), TPTC-29 (14.50 %), GC-3 (12.00 %), Pant lobia-3, (9.00 %), MC-17-2 (8.50 %), while minimum disease incidence was reported in genotype Pant Lobia-4 (5.00 %). During *Kharif*-2021 the maximum disease incidence was noticed in genotype CPD-311 (36.50%), followed by KBC-13 (35.00 %), TC-1901 (35.00 %), KBC-11 (32.00 %), GC-1712 (30.50 %), DC-15 (28.00 %), PCP 1124-1 (25.50 %), GC-1602 (22.00 %), PCP-0306 (20.50 %), CPD-313 (16.00 %), KBC-9 (19.00 %), PGCP-70 (15.00 %), TPTC-29 (13.50 %), RC-101 (13.00 %), PGCP-69 (12.50 %), GC-3 (12.00 %), MC 17-2 (10.50 %) and Pant lobia-3, (8.50 %), while minimum disease incidence was recorded in genotype Pant Lobia-4 (3.50 %).

Among the three year mean data the results indicates that none of the entry was found free from dry root rot. Maximum percent diseases incidence was recorded in genotype CPD-311 (44.50 %), followed by PGCP-70 (33.67 %), TC-1901 (33.17 %), KBC-13 (31.83 %), KBC-11 (31.67 %), DC-15 (28.83 %), PCP 1124-1 (23.17 %), PCP-

0306 (21.33 %), GC-1602 (20.50 %), KBC-9 (19.17 %), CPD-313 (16.83 %), PGCP-70 (16.00 %), PGCP-69 (15.67 %), RC-101(15.33 %), TPTC-29 (13.50 %), GC-3 (13.00 %), MC-17-2 (9.67 %) and Pant lobia-3 (9.17 %), while minimum disease incidence was recorded in genotype Pant Lobia-4 (4.50 %).

Table-1: Percent incidence of dry root rot among cowpea genotypes inoculated artificially.

Entry	Time taken for the appearance of first symptoms of dry root rot	(%) Incidence Disease 30 days after inoculation *			
		2019	2020	2021	Mean
GC-3	4	15.00	12.00	12.00	13.00
TPTC-29	5	12.50	14.50	13.50	13.50
RC 101	4	17.50	15.50	13.00	15.33
KBC-9	5	20.00	18.50	19.00	19.17
Pant Lobia-3	18	10.00	9.00	8.50	9.17
DC-15	3	30.00	28.50	28.00	28.83
Pant Lobia-4	15	5.00	5.00	3.50	4.50
PCP-0306	4	22.50	21.00	20.50	21.33
MC 17-2 (KBC-12)	12	10.00	8.50	10.50	9.67
CPD-311	3	47.50	49.50	36.50	44.50
KBC-13	4	32.50	28.00	35.00	31.83
CPD-313	6	17.50	17.00	16.00	16.83
PGCP-69	7	17.50	17.00	12.50	15.67
PCP 1124-1	4	22.50	21.50	25.50	23.17
KBC-11	6	32.50	30.50	32.00	31.67
GC-1602	7	20.00	19.50	22.00	20.50
TC-1901	4	32.50	32.00	35.00	33.17
GC-1712	5	35.00	35.50	30.50	33.67
PGCP-70	8	17.50	15.50	15.00	16.00
SEm(±)		**3.22**	**2.75**	**2.55**	**1.30**
C.D. at 5%		**9.13**	**8.20**	**7.64**	**3.75**

*Mean of three replications

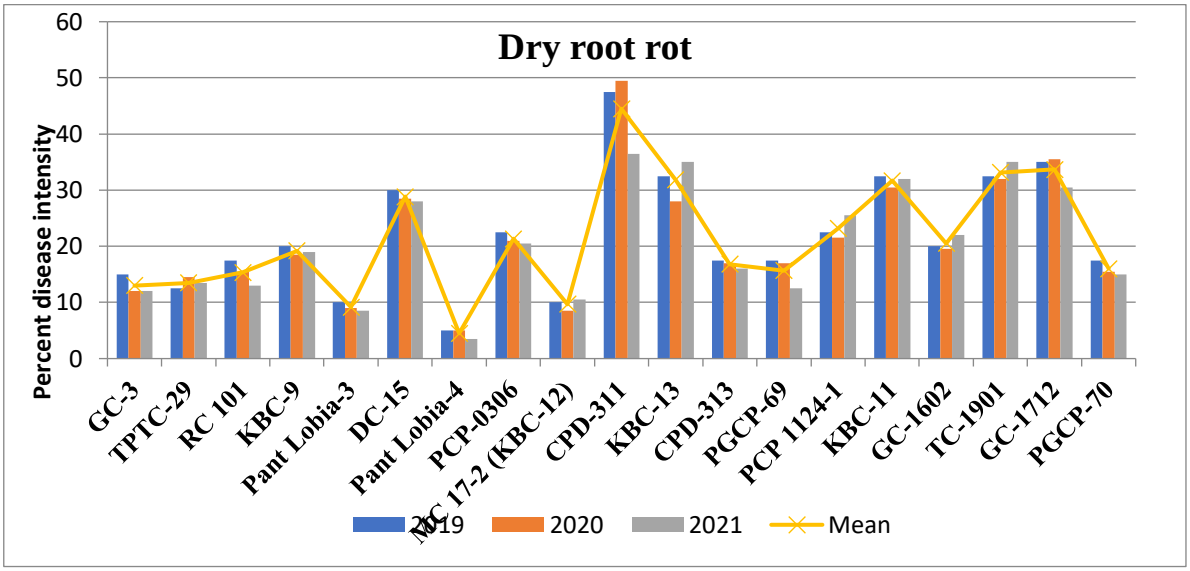
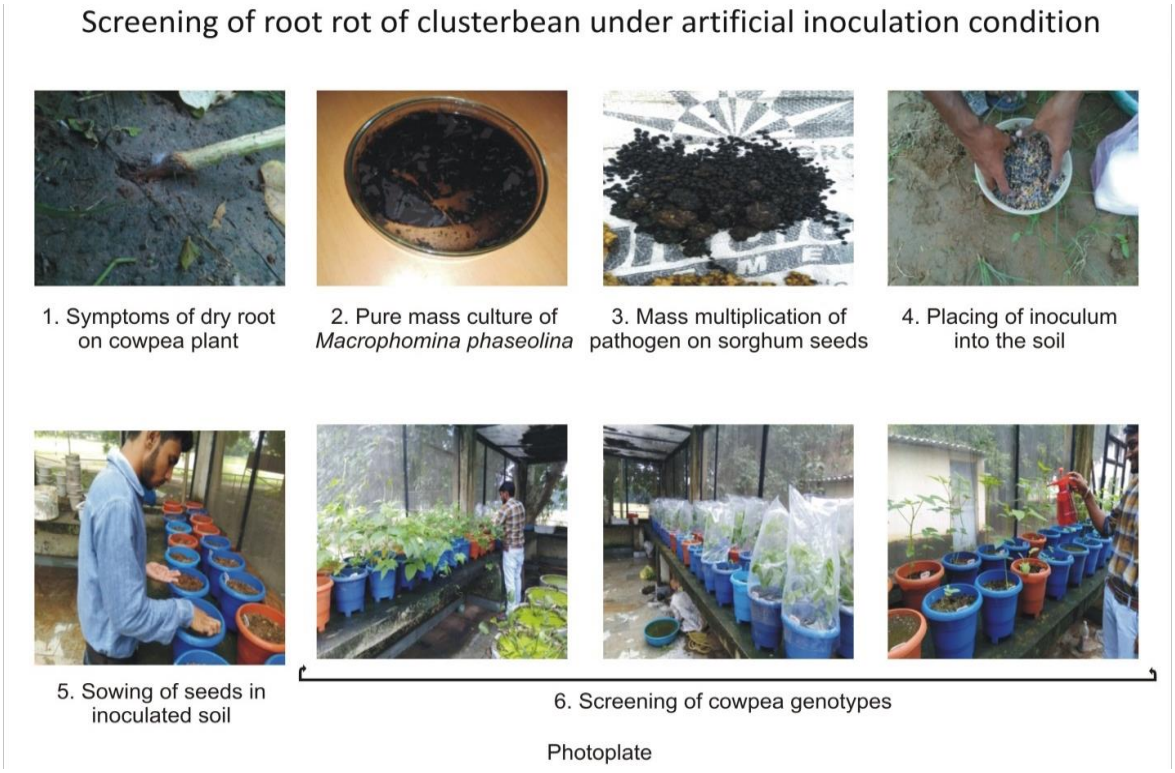


Fig-1: Screening against dry root rot incidence through artificial inoculation methods



Disease severity of dry root rot among cowpea genotypes.

Disease severity index was significant for different genotypes under artificial inoculation condition. The various cowpea cultivars were categorized under various usceptibility and resistant class through this limiting factor. Cultivar Pant Lobia-3, Pant Lobia-4, MC-17-2 (KBC-12) were moderate resistant to the pathogen *Macrophomina phaseolina.*

During *kharif*-2019 the higher disease severity index was seen in genotype KBC-11 and GC-1712 (91.70 %) followed by TC-1901 (83.30 %), KBC-13 (83.30 %), CPD-311 (75.00 %), DC-15 (66.70 %), PCP 1124-1 (20.00%), PCP-0306 (18.30 %), GC-3 (8.33 %), RC-101, KBC-9, CPD-313, PGCP-69 (6.67 %) respectively TPTC-29 (5.00 %), GC-1602 (5.00 %), Pant lobia-3 (3.33%), Pant Lobia-4, PGCP-70 (1.67 %), while lower disease severity index was secured in genotype MC-17-2 (KBC-12) (0.00 %). During *kharif* 2020 the lower

disease severity index was secured in genotype KBC-11 and GC-1712 (91.70%), followed by TC-1901 (83.30 %), CPD-311 (75.00 %), KBC-13 (66.70 %), DC-15 (66.70 %), PCP 1124-1 (13.30 %), PCP-0306 (8.33 %), KBC-9, CPD-313 (6.67 %), TPTC-29 and RC-101 (5.00 %), GC-3, Pant lobia-3, PGCP-69, GC-1602, PGCP-70 (3.33 %), MC-17-2 (KBC-12) (1.67 %), percent severity of disease was observed in genotype Pant Lobia-4, (0.00%). During *kharif* 2021 the highest disease severity index was recorded in genotype GC-1712 (91.67 %), followed by KBC-11 (83.33 %), CPD-311 (75.00 %), TC-1901 (75.00 %), KBC-13 (66.67 %), DC-15 (58.30 %), PCP-0306 (33.33 %), PCP 1124-1 (28.33 %), KBC-9, GC-1602 (6.67 %), RC-101, Pant lobia-3 (5.00 %), while lower disease severity index was noted in genotype GC-3, TPTC-29, Pant Lobia-4, CPD-313 and PGCP-70 (1.67 %).

The three year pooled mean data reveals that none of the entry was found free from dry root rot. Maximum disease severity index was recorded in genotype GC-1712 (91.69 %), followed by KBC-11 (88.91 %), TC-1901 (80.53 %), CPD-311 (75.00 %), KBC-13 (72.22 %), DC-15 (63.90 %), PCP 1124-1 (20.54 %), PCP-0306 (19.99 %), KBC-9 (6.67 %), RC-101(5.56 %), CPD-313 and GC-1602 (5.00%), GC-3 (4.44 %), Pant lobia-3, PGCP-69 and TPTC-29 (3.89 %), PGCP-70 (2.22 %), while lower disease severity index was noted in genotype Pant lobia-4 and MC-17-2 (KBC-12) (1.11 %) respectively. On the basis of data recorded disease severity eleven genotypes belong to moderately resistant, two genotypes are belong to moderately susceptible six genotypes are belong to highly susceptible class.

Table-2: Percent severity index and reaction genotypes against DRR diseases

Entry	Disease severity index %				Plant rating	Susceptibility resistance class
	2019	2020	2021	Mean		
GC-3	8.33	3.33	1.67	4.44	1	MR
TPTC-29	5.00	5.00	1.67	3.89	1	MR
RC 101	6.67	5.00	5.00	5.56	2	MR
KBC-9	6.67	6.67	6.67	6.67	2	MR
Pant Lobia-3	3.33	3.33	5.00	3.89	1	MR
DC-15	66.7	66.7	58.3	63.90	5	HS
Pant Lobia-4	1.67	0.00	1.67	1.11	1	MR
PCP-0306	18.3	8.33	33.33	19.99	3	MS
MC 17-2 (KBC-12)	0.00	1.67	1.67	1.11	1	MR
CPD-311	75.0	75.0	75.00	75.00	5	HS
KBC-13	83.3	66.7	66.67	72.22	5	HS
CPD-313	6.67	6.67	1.67	5.00	1	MR
PGCP-69	6.67	3.33	1.67	3.89	1	MR
PCP 1124-1	20.0	13.3	28.33	20.54	3	MS
KBC-11	91.7	91.7	83.33	88.91	6	HS
GC-1602	5.00	3.33	6.67	5.00	1	MR
TC-1901	83.3	83.3	75.00	80.53	6	HS
GC-1712	91.7	91.7	91.67	91.69	6	HS
PGCP-70	1.67	3.33	1.67	2.22	1	MR

Entry	Disease severity index %				Plant rating	Susceptibility resistance class
	2019	2020	2021	Mean		
SEm(±)	5.84	7.22	9.10	2.67	NS	NS
C.D. at 5%	16.82	20.81	26.21	7.71	NS	NS

***Mean of three replication**

0-6 scale in which 0 (Highly Resistant) =0, 1 (Moderately Resistant) = 1-5, 2 (Moderately Resistant) = 6-10, 3 (Moderately Susceptible) = 11-25, 4 (Moderately Susceptible) = 26-50 , 5 (Highly Susceptible) = 51-75, 6 (Highly Susceptible) = 76-100 modify person scale (1997).

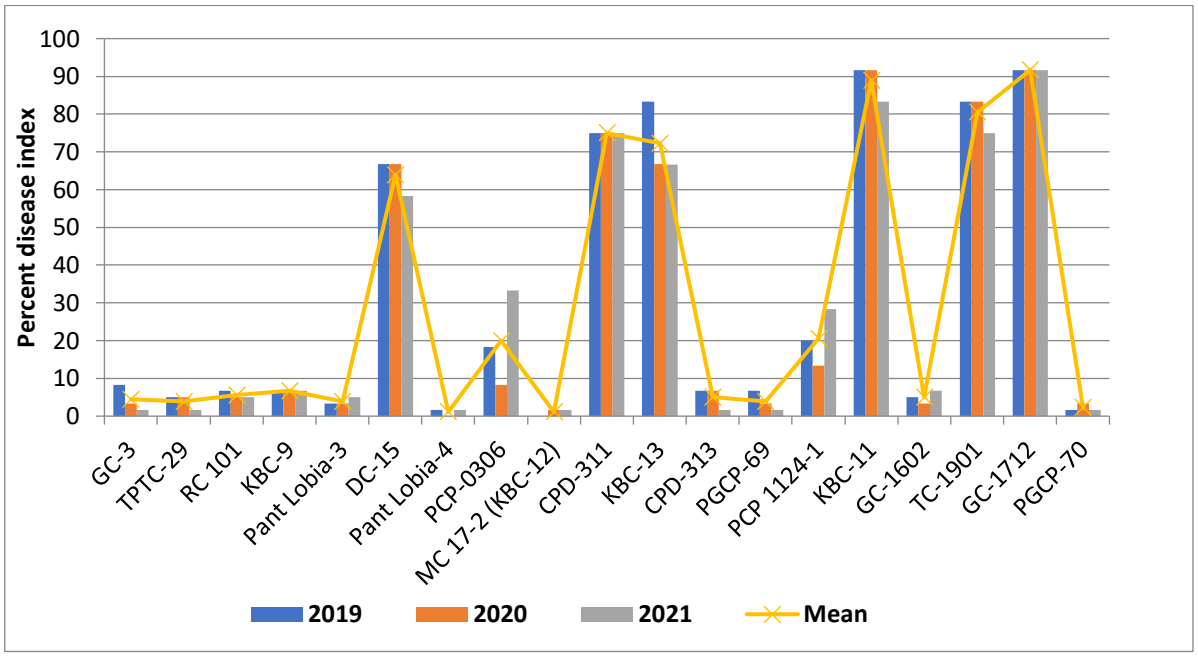


Fig-2: Influenced of cowpea genotypes against dry root rot disease index under artificial inoculation condition.

4. Discussion

Dry root rot is incited by a polyphagous pathogen Macrophomina, and is soil loving pathogen with varied host scale as well as global dispersion (Olaya *et al.*, 1996 and Aboshosha *et al.*, 2007). It causes severe disease symptoms on economically important crops such as legume and vegetables kour *et. al.*, (2012), Kumar *et. al.*, (2017). After infection the pathogen passes through the vascular system of plant and result in obstruction of water and nutrient movement of the plant. The seed and soil borne nature of dry root rot pathogen is challenging for its control and also can remain alive for several year in soil. In developing countries, the extensive use of fungicides is not economical and reliable to manage the disease at farmers' level. In this context using disease resistance varieties is the foremost option to control the dry root rot. Jaylaxmi *et. al.*, (2008), Sharma *et al.*, (2012) and lekhraj *et. al.*, (2012) also screened the legume crop against dry root rot pathogen. Efficient method for controlling the soil borne disease of crop plant is feasible through resistant varieties which are most economical cheap and eco-friendly for marginal poor farmers in contrast to chemicals. For this identification of sources of resistance is the first step. In the present study nineteen cowpea genotypes were evaluated using artificial inoculation method. Among these genotypes eleven genotypes are moderate resistant in reaction, two genotypes are moderate susceptible and six genotypes are highly susceptible.

5. Conclusion

This research presented that the a restricted evaluation of artificial inoculation technique with nineteen cowpea genotypes in northern Madhya Pradesh. Incidence and varied significant rainfed cowpea cropping season. Further more testing of various inoculation methods and their comparisons will be obligatory to find cowpea genotypes unaffected by *Macrophomina phaseolina.* Among all the cowpea genotypes used in present investigation diseases severity index none of the entry was found free from dry root rot, however eleven genotypes are belong to class moderately resistant, six genotypes are belong to moderately susceptible two genotypes are belong to highly susceptible.

Recommendation:

Molecular evaluation of the *Macrophomina phaseolina* will help to identify the variation within the causal agent of the disease. It also enhance the selection and recognition of suitable resistant cowpea genotypes. Resistant source may be useful in the breeding programme for the identification of high yielding and disease resistance genotypes.

Objectives

- Screening of obtainable genetic stocks (local land races, advance and non-native germplasm) in net house is the first step towards the resistance breeding programme scuitftly.
- The major objective of screening is to acquire a more even distribution of disease among genotypes.
- The success of artificial screening is mainly dependent on, an aqeuate amount of inoculum and the right method or procedure is to be followed for inoculation.

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