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Pot screening of promising chickpea varieties against collar rot caused by *Sclerotium rolfsii* (Sacc.) under artificial inoculation in net-house

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Abstract
The experiment was conducted during *Rabi*, 2019-20 to find to Disease resistance reaction of promising cultivar of chickpea against collar rot of chickpea caused by *S. rolfsii* under artificial inoculation in pot experiment. Ten promising cultivar/genotypes viz., RKG-13-515, GNG-1958, RKG-18-1, JG-14, RVG-201, GNG-1469, GNG-2144, JG-16, CSJ-515(c) and RKG-13-515 (1) were sown in the pots under artificial inoculated condition for screening. Out of the 10 entries screened with soil inoculated in pots, no one varieties show resistance against collar rot disease under artificial inoculated condition. Minimum pre-emergence seed rotting (23.33%) and post emergence seedling mortality (78.26%) were observed in GNG-1958. This shows a high level of aggressiveness of the pathogen or relatively narrow diversification of genetic material under study.

Keywords: Chickpea, collar rot, screening, *S. rolfsii*, soil inoculated and promising cultivar

Introduction
Chickpea is one of the vital sources of protein required for humans it has various health benefits. Chickpea is a legume plant that grows in subtropical and temperate regions. It is cultivated mainly on lands under rainfed condition in *Rabi* season (Shiyani *et al*., 2001) [14]. Two distinct market types *i.e.*, desi and kabuli are recognized (Pundir *et al*., 1985) [9]. Chickpea is an important grain legume providing an enormous source of minerals, fibers, and proteins both for humans and animals (Varol *et al*., 2020) [16]. On the other hand, chickpea plays an important role in the improvement of soil fertility by fixing atmospheric nitrogen. It meets 80 per cent of nitrogen necessity from symbiotic nitrogen fixation, and can fix up to 140 Kg N/ha from air, and adds a large amount of residual nitrogen for subsequent crops. It also adds plenty of organic matter which improves soil health and fertility. Because of its deep taproot system can resist in drought conditions by extracting water from deeper layers in the soil profile (Kashiwagi *et al*., 2005; Krishnamurthy *et al*., 2003) [5, 6].
India ranks first in conditions of chickpea production and consumption in the world. About 65 per cent of the global area with 68 per cent of global production is contributed by India. Despite the high total production and more nutritive value, productivity of chickpea was low due to many biotic and abiotic constraints. Among the biotic constraints of chickpea soil borne diseases such as Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) are the major limiting factors in chickpea production. Chickpea diseases may cause yield losses up to 100% depending on time of infection. *Sclerotium rolfsii* is an economically important pathogen with a wide host range of at least 500 species in 100 families. The characteristic symptoms of the disease contain rapid plant wilting with dark brown lesions at the stem base, which later on girdles the main stem. Infected plant tissues as well show a white mycelial growth that frequently radiates over the soil surface (Acabal *et al*., 2019) [1]. Collar rot caused by *Sclerotium rolfsii* is the major limiting constrain in chickpea cultivation, which causes significant yield losses up to 45% (Sarkar *et al*., 2014) [11]. Dry root rot and collar rot are emerging as a major threat to chickpea production due to drastic climate change (Pande *et al.* 2010) [7]. It was revealed that the pathogen attacks the crop at the seedling stage, causing severe yield losses in chickpea growing areas (Javaid and Khan, 2016 and Tarafdar *et al*., 2018) [4, 15]. Affected seedlings turn yellow and die. The seedlings generally collapse and show rotting at the collar region and below. *S. rolfsii* control has met with minimal success. This may be due to the abundant

growth of the pathogen and having the ability to produce a large number of sclerotia that may continue in the soil for several years (Sennoi *et al*., 2013) [12]. As the genetical resistance is not available in chickpea crop till now, the only practicable and cost-effective control for such a devastating soil-borne pathogen is selection of cultivars. Therefore, the present study was carried to screen the chickpea promising cultivar against *S. rolfsii* for the identification of resistant sources in pot house under artificial inoculation conditions.

Materials and Methods

Collection, isolation, pathogenicity and identification of *S. rolfsii*: Infected plants which showing typical collar rot symptoms were collected during month of October to December, 2018 from the chickpea fields of Agriculture Research Station, Ummedganj- (Kota) brings to laboratory for further studies. Isolation of fungus was carried through standard tissue isolation through infected plant parts and the pure culture of fungus was obtained by further growing culture and following hyphal tip culture under aseptic conditions were maintained on PDA slants at 4±1°C for further studies. Pathogenicity was proved through soil inoculation. Basis on culture characteristics fungus identified as *S. rolfsii*. Further, the identification of pathogen was confirmed from Indian Type of Culture Collection, Division of Plant Pathology, IARI, New Delhi (Ref. No. PP/3260; Date- 25/03/2019).

Soil sterilization: For pot study the soil was sterilized by using formaldehyde by the following procedure. For this raised soil bed was prepared and watered the soil up to saturation level and left undisturbed for two days. After two days the soil was moistened by 4% formaldehyde solution (40 ml formaldehyde per liter of water) up to saturation level and covered by polythene sheet and kept undisturbed for five days. Polythene sheet was removed after five days and soil was exposed to open for seven days to remove the traces of formaldehyde present in soil. This soil was filled to the disinfected pots to carry out further studies.

Screening in pots: The sterilized soil, sand and FYM were mixed in 1:1:0.5 proportion (w/w basis) and filled in disinfected cemented pots. 10 gm mass culture of *S. rolfsii* grown on sorghum seeds was added to upper 15 cm layer of soil in pots and mixed thoroughly. Healthy seeds of selected 10 genotypes *viz.*, RKG-13-515, GNG-1958, RKG-18-1, JG-14, RVG-201, GNG-1469, GNG-2144, JG-16, CSJ-515(c) and RKG-13-515 (1) were sown in the pots replicate thrice for screening. Moisture content in soil was maintained to field capacity by adding required amount of water when needed.

Observation recorded: The percentage seed germination, pre-emergence seed rot and post-emergence seedling mortality were calculated by the formulae were made regularly at 10 days interval. Disease reaction was made according to IIPR collar rot rating scale (Shirsole *et al.*, 2018) [13].

a.) Germination (%) = $\frac{\text{Number of seed germinated}}{\text{Total number of seed sown}}$X100

b.) Pre-emergence seed rotting %

(PESR) = $\frac{\text{Number of seed not germinated}}{\text{Total number of seed sown}}$X100

c.) Post-emergence seedling mortality %

(PESM) = $\frac{\text{Number of seedling died}}{\text{Total number of seedling}}$X100

IIPR collar rot rating scale (Shirsole *et al.*, 2018) [13]

IIPR collar rot rating scale

S. No.	Reaction	Percent Mortality	Score
1.	R- Resistant	< 10	1
2.	MR- Moderately Resistant	10-20	2
3.	MS- Moderately Susceptible	20-30	3
4.	S- Susceptible	30-40	4
5.	HS- Highly Susceptible	> 40	5

Statistical analysis of experimental data: Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) for both as well as laboratory and pot experiments as suggested by Panse and Sukathme (1985) [8].

Results and Discussion

Screening of chickpea varieties against collar rot under artificial soil inoculation method in net house: Experiment was conducted to screened chickpea varieties (desi) against collar rot under artificially inoculated condition in net house and data were recorded in Table-1, illustrated in Fig.-1 and Plate-1. Ten genotypes/varieties *viz.*, RKG-13-515, GNG-1958, RKG-18-1, JG-14, RVG-201, GNG-1469, GNG-2144, JG-16, CSJ-515(c) and RKG-13-515 (1) were sown in pots for screening against collar rot disease. Observations on percent collar rot incidence were documented at 10 days interval. It is evident from data presented in Table-1, that pathogen caused both pre-emergence seed rot (23.33 to 70%) and post emergence seedling mortality (78.26 to 100%) up to 30 DAS. Out of the 10 entries screened with soil inoculated in cemented pots, no one varieties show resistance against collar rot disease under artificial inoculated condition. Minimum pre-emergence seed rotting (23.33%) and post emergence seedling mortality (78.26%) were observed in GNG-1958. This shows a high level of aggressiveness of the pathogen or relatively narrow diversification of genetic material under study. Such finding correlated with Amule *et al.*, (2014) reported that among 88 chickpeas desi genotype GNG 1958 was found resistant to disease whereas, 13 entries *viz.*, NDG 9-21, PG 97030, BG 3004, JG 14-11, H 04-68, PG 054, BGD 1058, GJG 0724, RSG 931, JG1307, GJG 0504, JG 14-110, H05-24 were moderately resistant. Among Kabuli types, two entries *i.e.*, GNG 1969, BG 2086 were resistant and 9 as moderately resistant (IPCK 2005-23, Phule G 0027, JGK 2003-304, IPCK 02, BG 3001, MNK 1, BG 3000, Vihar, HK 06-168). Gurakhede *et al.*, (2015) reported that in a field screening of 284 chickpea germplasm accessions against collar rot, 9 were found free from disease and 29 exhibited < 10 per cent mortality due to collar rot. Sab *et al.*, (2018) [10] performed a study for detect host plant resistance against the collar rot disease of chickpea, two-hundred and six entries were screened under field conditions and promising entries under greenhouse conditions (Artificial inoculation) against collar rot and seven fungicides were used as seed dressing to manage the collar rot disease of chickpea. For confirmation of promising entries which showed resistant reaction in field conditions, eight entries were selected *viz.*, Vishal, BG-256, HIR-55, BBG-1, HIR-60, BBG-2, KAK-2, and HIR-70 were

sown in the pots along with Annigeri-1 as susceptible check. Among them, two entries *viz.*, BG256 and KAK-2 were free from infection (0%) whereas HIR55, BBG-2 HIR-60 BBG-1 HIR-70 showed 8, 15, 20, 22 and 33 per cent infection, respectively. Vishal was most susceptible with 53% infection compared to 60% infection in susceptible check (Annigeri-1). Shirsole *et al.*, (2018) [13] Mass culture of the pathogen was prepared on wheat grains media and inoculated in collar zone of chickpea plant, 15 days after sowing. Out of 185 chickpea entries only 5 entries viz., GNG 2331, JG 2016-9605, IPC 2012-98, RVSSG-38 and GL 12003 exhibited moderately resistant response while, the remaining were susceptible to highly susceptible for collar rot of chickpea.

Table 1: Screening of chickpea varieties against collar rot under artificial soil inoculation in net house.

S. No.	Varieties	Germination %	PESR %	PESM %	Reaction
1.	RKG-13-515	73.33 * (58.91) **	26.67 (31.09)	81.82 (64.76)	HS
2.	GNG-1958	76.67 (61.12)	23.33 (28.88)	78.26 (62.21)	HS
3.	RKG-18-1	40.00 (39.23)	60.00 (50.77)	100.00 (90.00)	HS
4.	JG-14	46.67 (43.09)	53.33 (46.91)	92.86 (74.50)	HS
5.	RVG-201	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	HS
6.	GNG-1469	60.00 (50.77)	40.00 (39.23)	88.89 (70.53)	HS
7.	GNG-2144	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	HS
8.	JG-16	73.33 (58.91)	26.67 (31.09)	81.82 (64.76)	HS
9.	CSJ-515-[C]	60.00 (50.77)	40.00 (39.23)	83.33 (65.91)	HS
10.	RKG-13-515-(1)	50.00 (45.00)	50.00 (45.00)	80.00 (63.43)	HS
S Em. ± =		0.69	0.68	0.83	
C.D. at 0.05% =		2.05	2.02	2.45	

*Average of three replications; **Figures in parentheses are Arc sine transformed values. **PESR**= Pre-emergence seed rotting, **PESM** = Post emergence seedling mortality.

Plate 1: Response of chickpea varieties against collar rot under artificial soil inoculation in net house.

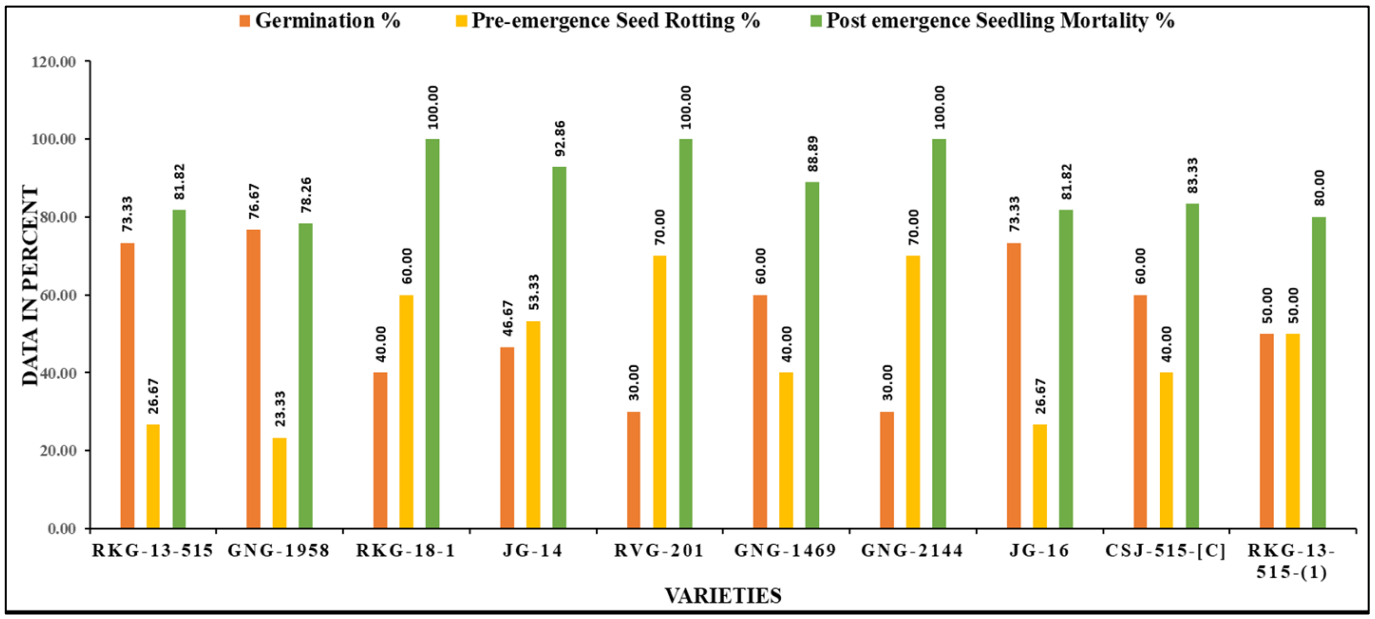

Fig 1: Screening of chickpea varieties against collar rot under artificial soil inoculation in net house.

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