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Survey, occurrence and standardization of pathogenicity protocol of black scurf of potato caused by *Rhizoctonia solani*

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Abstract
Potato (*Solanum tuberosum* L.) is one of the most important annual, herbaceous dicotyledonous and vegetatively propagated crop belong to family Solanaceae. Black scurf reduce tuber quality, marketability and cause a serious disease of potato worldwide. In India responsible for 10-25% yield loss, yield losses may reached up to 50% in severely affecting potato crops. The most appropriate symptoms of the black scurf were observed as sclerotial masses on tubers after harvesting of crop. Surveyed results indicated in the prevalence of disease at all the surveyed area *viz.*, Karmullapur, Prempur, Tilyani, Shivpuri, Dheerpur, Badanpur, Saidpur, Nagla, Budrai, Hazratpur, Sawai, Dandaniyapur, Bibapur and Mugnisapur. The maximum percent disease incidence and disease severity was recorded in Firozabad district (36.50 and 17.07% respectively), followed by Agra (28.50 and 15.52% respectively), while minimum percent disease incidence and disease severity was recorded in Etawah district (17.00 and 8.97% respectively). Among the villages, maximum percent disease incidence and disease severity was recorded in Budrai village of Firozabad district (37.00 and 17.30% respectively) followed by Hazratpur village of Firozabad district (36.00 and 16.85% respectively) while minimum percent disease incidence and disease severity was recorded in Nagla village of Etawah district (15.00 and 8.80% respectively). The data pertaining to disease incidence and disease severity of seven isolates, RS-5 showed high disease incidence and disease severity (93.10 and 44.68% respectively) followed by RS-3 (92.30 and 42.46% respectively) while RS-13 showed least virulence and recorded minimum disease incidence and severity (82.14 and 33.53% respectively) followed by RS-7 (85.18 and 35.72% respectively) hence, the most virulent isolate (RS-5).

Keywords: Survey, pathogenicity, *Solanum tuberosum*, black scurf, disease incidence and disease severity

Introduction
Potato (*Solanum tuberosum L.*) is one of the most important annual, herbaceous dicotyledonous and vegetatively propagated crop belong to family Solanaceae. It can also be propagated through botanical seed known as true potato seed (TPS). The potato is a modified stem developed underground on a specialized structure called stolon. It is fourth largest grown crop after rice, wheat and maize in the world. Next to cereals, potato is the only crop which could supplement the need of the food of the country (Das *et al*., 2000) [7]. It is economical food containing less energy but nutritional high quality of protein, essential vitamins and minerals including trace elements and provide a source of low cost energy to the human diet (Mehdi *et al*., 2008) [14]. Potato also contain a good amount of essential amino acid like leucine, tryptophane, isoleucine and main source of starch and carbohydrate etc. It is used for several industrial purposes such as for the production of starch and alcohol, processed into several products like- chips, French fries, cubes, granules and canned products. The vegetative green parts are used as fodder and tubers are chiefly used as vegetable. The average composition of potato tuber per 100g edible portion is moisture (74.7g), protein (2.0g), fat (0.1g), carbohydrates (22.6g), energy (85 calories), calcium (13mg), phosphorus (40mg), iron (0.70mg), carotene (24µg), vitamin A (40 units), thiamine (0.11mg), riboflavin (0.01mg) and vitamin-C (12mg) (Das *et al*., 2000) [7].

Potato production occupy total area of 1, 50,864 hectares with the production of 38 million tons worldwide. China is the largest producer of potatoes worldwide with 99,205,600 tons followed by India with 48,605,000 tons in 2019 (FAOWSTAT, 2020). Together, both China and India contribute 38% to the world's total production. In India, the potato is not primarily a rural staple but also a cash crop that provides significant income to farmers.

Among the India, Uttar Pradesh is the largest producer of potato with 14,755,000 tons which occupy 30.40% to all over India which increased by 6-8 per cent as farmers had earned higher income in potato in 2020 than in the previous year, (Anonymous, 2020) [3]. Among the Uttar Pradesh, Kannauj is a largest producer of potato with 956601.30 tons with the productivity of 25.43 tons / ha.

Potato is commonly known as disease oriented problematic crop throughout the world. Potato production is threatened by many factors in which diseases have maximum importance, which are caused due to Nematodes, Fungi, Bacteria, viruses etc. Among all these pathogens, fungi caused many diseases such as late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), wart disease of potato (*Synchytrium endobioticum*), Phoma leaf spots (*Phoma andigena var. andina*), Pink rot (*Phytophthora erythroseptica*), Charcoal rot (*Macrophomina phaseolina*) black scurf (*Rhizoctonia solani*) etc. among these diseases black scurf is one of the serious problem of potato caused by *Rhizoctonia solani* (perfect stage- *Thanetophorus cucumeris*). *Rhizoctonia* stem canker is one of the destructive and soil borne disease of potato and *R. sola*ni is a complex pathogen with wide host range in all over potato growing countries. Black scurf reduce tuber quality, marketability and cause a serious disease of potato worldwide. In India responsible for 10-25% yield loss (Sharma, 2015) [21], yield losses may reached up to 50% in severely affecting potato crops.

Black scurf was originally described by Julius Kühn from potato in 1858 (Kühn 1858) [13]. Rhizoctonia (the Greek word Death of roots) canker commonly called black scurf is one of the oldest and most common infection of potato stems and stolons below the soil surface and results in stem canker whereas sclerotia produced by the pathogen on tubers are referred to as black scurf. Black scurf disease symptoms can be found on all underground parts of the plant at different times during the growing season. Disease is pronounced when black sclerotia cover the tuber surface. The disease causes defacing of tuber with the deposition of sclerotia. As a result, farmers have to bear 5-7% economic loss (Shekhawat, *et al*., 1993 and Singh and Shekhawat 1994) [24]. Under minimum disease severity it just lower down the market price but may not reduce the yield. Under severe conditions, when sclerotia cover more than 50% area hinders in germination of tubers, if get germinated there will be poor plant growth and leads to low yield. The disease is a potential threat to the fast developing seed potato cultivation in India plains. Soil-borne inoculums of *R. solani* is the main cause of black scurf, it is the serious and most commonly observed disease (Ahmed *et al.*, 1995; Khan *et al.*, 1995) [11] with the characteristic symptoms of black scurf (dark brown to black coloured hard masses of sclerotia, irregularly shaped and superficial, varying from small, flat, barely detectable blotches to large and raised lumps adhering tightly to the skin) on potato tubers and stem canker are the result of *Rhizoctonia* disease complex in potato (Tsror, 2010) [25]. It also contributes to eyes germination inhibition, sprouts killing, stem, stolon and root damage. The hyphal of *Rhizoctonia*, attacks developing sprouts when the soil is cool (50-59°F) and moist. Symptoms on developing sprouts appear as reddish-brown, discolored areas, and the growing point of severely infected sprouts is often killed (DPP 2000). It was observed that the differences in the incidence of stem canker, stolon canker and black scurf were dominated by the effect of inoculums on seed tubers at planting (Simons and Gilligan 1997) [23]. It is distributed in India in different regions at different levels of severity and is a major disease problem in fields where potato is cultivated year after year in the same field **(**Khurana *et al.*, 1998; Arora, 2011 and 2012) [12]. Although, this disease do not affect the yield quantitatively but deteriorate the quality and acceptability of tubers for seed adversely affecting the market price of the table potatoes. After harvest of crop, sclerotia that can be seen on potato seed tubers do no wash off with water. The fungus maintained its life cycle and transmitted by sclerotia on the tubers and within the soil or plant residues. With adequate temperature and soil moisture, the sclerotia germinate and penetrate stems, roots and stolons. Development of sclerotia on tubers is pronounced in poorly drained soils. The management of *R. solani* is very difficult due to its soil-borne nature. The fungus is present in most of the soils. Once it becomes established in a field, it remains viable there indefinitely (Agrios, 2005) [1].

Materials and Methods

Survey and collection of diseased materials from major potato growing area of U.P.

An intensive roving survey was conducted during *Rabi* 2018-19 and 2019-20 at the farmer's field of the seven district of Uttar Pradesh (Kannauj, Mainpuri, Farrukhabad, Etawah, Firozabad, Agra and Kanpur Dehat) and collected the sample from each district to find out the incidence of black scurf of potato. In each district, a minimum of two villages and in each village two fields were examined. A total of 28 fields were surveyed at the time of harvesting. Five place were selected for each field and 20 tubers were examined and black scurf infected and healthy tubers were counted. Disease incidence was determined by using following formula-

$$\text{Disease Incidence} = \frac{\text{Number of infected tubers}}{\text{Total tuber observed}} \text{X100}$$

$$\text{Disease Severity} = \frac{\text{Sum of Individual rating}}{\text{Number of tuber examined x Maximal disease rating}} \text{X100}$$

Tubers were harvested at plant senescence and washed then each tuber was scored from 0 to 5 disease rating scale according to a visual tuber surface cover to calculate the disease severity of black scurf of potato developed by Ahmad *et al.* (1995) [2]. Whereas, 0 = no symptoms on potato tubers; 1 = less than 1% tuber area affected; 2 = 1-10% tuber area affected; 3 =11-20% tuber area affected; 4 =21-50% tuber area affected; 5 = 51% or more tuber area affected.

Table 1: List of surveyed districts and their code of major potato growing area of UP.

Surveyed District	Villages	Code of isolates
Kannauj	Karmullapur	RS-1
	Prempur	RS-2
Mainpuri	Tilyani	RS-3
	Shivpuri	RS-4
Farrukhabad	Dheerpur	RS-5
	Badanpur	RS-6
Etawah	Saidpur	RS-7
	Nagla	RS-8
Firozabad	Hazratpur	RS-9
	Budrai	RS-10
Agra	Sawai	RS-11
	Dandaniyapur	RS-12
Kanpur Dehat	Bibapur	RS-13
	Mugnisapur	RS-14

Isolation, purification, identification and maintenance of *Rhizoctonia solani* isolates

Isolation

The diseased tubers showing small crust like bodies, having very dark or black colour sclerotia were collected during survey from various locations and used for isolation in laboratory. The infected tubers were washed several time to romove soil adhering to potato. Sclerotia were cut along with some potato skin with the help of sterilized scalpel. After surface sterilization with 1.0% sodium hypochloride solution for 30 seconds, sclerotia were rinsed three times in sterile distilled water to eliminate surface contaminations. Sclerotia were allowed to dry on sterile tissue papers. Twenty ml of the PDA medium was poured in sterilized petri plates and allowed them to solidify. The pieces were then placed on the poured medium under aseptic condition in a laminar air flow. The plates were sealed with Parafilm to avoid contamination and then incubated at 25 ± 2 ^{O}C and checked regularly for further growth.

Purification of pathogen

Purification of isolated fungus of each isolate was carried out by using hyphal tip technique as described by Dhingra and Sinclair (1985) [8]. Purified test pathogen was used for identification.

Identification of pathogen

The isolates collected from tubers were initially identified as *Rhizoctonia* by visual observation of their hyphal characteristics on potato dextrose agar (PDA) medium by Ogoshi (1987) [19]. Hyphae of *Rhizoctonia* have right-angle branches. Their identity was confirmed by hyphal staining with 0·05 per cent trypan blue in lactophenol (Meyer *et al*., 1998) [15] followed by light microscopy under a compound microscope to determine their hyphal morphology. *Rhizoctonia solani* produced dark brown to black sclerotia after seven days of incubation on PDA. Hyphal staining showed pathogen had hyphae characteristic of *R. solani* with typical right-angle branches. (Pandit, 2017) [20].

Maintenance and preservation of culture

Pure cultures of different isolates were maintained on PDA slants by sub-culturing it at 30 days interval. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored at 7.5 ± 1 °C in a refrigerator.

Pathogenicity test of *Rhizoctonia solani*

In order to determine the pathogenic nature of the fungus, the pathogenicity test of the isolated fungus was made on the healthy tubers of the plants. The pathogenicity of the organism was tested according to Koch's postulates. For inoculation purpose, experiments were carried in seven pots. The inoculum was prepared by growing pure culture of the isolated fungus on sand corn meal medium. The sterilised soil was inoculated with prepared culture of different isolates of the pathogen, pots were filled with inoculated soil and watering was done to provide adequate moisture. Normal seed of the host were firstly disinfected with 0.1% mercuric chloride solution for two minutes and then were rinsed with sterilised water, dried and sown in pots. One pot was also selected for control purpose and was treated equally except the inoculation with mycelial culture of pathogen. Pots containing plants were watered periodically to maintain sufficient moisture for proper growth of the plants and production of tubers. Affected tubers were counted after harvesting of tuber.

Pathogenicity test of the fungus was also done on the leaves and stem of the potato plants. Leaves and stem of the plants were thoroughly washed by spraying sterilised water with an atomizer. Leaves were slightly injured with the help of sterilised needle and inoculated with the suspension of fungus culture by spraying the inoculam with the help of an atomizer. The suspension was prepared by mixing sterilised water in pure culture. Some control plants were also selected for the purpose of comparison and they were treated equally except the inoculation with mycelial culture. After inoculation, these pots were kept under moist chamber for 48 hours by covering them with polythene bags. This was done in order to provide optimum humidity to the pathogen for causing infection. The uninoculated plants were also placed in moist chamber for 48 hours. All the plants were then removed to glass house benches and watched for the appearance of the disease symptoms.

Results and Discussion

Table-1, showed that, an extensive survey was conducted during Rabi 2018-19 and 2019-20 at the farmer's field of the seven districts of Uttar Pradesh *viz.*, Kannauj, Mainpuri, Farrukhabad, Etawah, Firozabad, Agra and Kanpur Dehat to find out the percent disease incidence and disease severity of black scurf of potato. The most appropriate symptoms of the black scurf were observed as sclerotial masses on tubers after harvesting of crop. Surveyed results indicated in the prevalence of disease at all the surveyed area *viz.*, Karmullapur, Prempur, Tilyani, Shivpuri, Dheerpur, Badanpur, Saidpur, Nagla, Budrai, Hazratpur, Sawai, Dandaniyapur, Bibapur and Mugnisapur. Table-2 indicated that maximum percent disease incidence and disease severity was recorded in Firozabad district (36.50 and 17.07% respectively), followed by Agra (28.50 and 15.52% respectively), while minimum percent disease incidence and disease severity was recorded in Etawah district (17.00 and 8.97% respectively). Among the villages, maximum percent disease incidence and disease severity was recorded in Budrai village of Firozabad district (37.00 and 17.30% respectively) followed by Hazratpur village of Firozabad district (36.00 and 16.85% respectively) while minimum percent disease incidence and disease severity was recorded in Nagla village of Etawah district (15.00 and 8.80% respectively). Similar findings was confirmed by earlier researchers Ahmad *et al*., (1995) [2] and Balali *et al.*, (1995) [6] the isolates of *Rhizoctonia* collected from the tuber of potato crops which were identified to anastomosis groups of the 301 multinucleate isolates of *R. solani* tested, 90% were AG-3, 7% were AG-4 and 2% were AG-5; 12 isolates were binucleate *Rhizoctonia spp*. Neha *et al.*, (2016) [18] survey was carried out to assess the occurrence of sheath blight disease incidence in major paddy growing areas of Cuddalore district of Tamil Nadu, India revealed the endemic nature of disease. Mughal *et al*., (2017) [16] twenty seven isolates of *Rhizoctonia solani* collected from different locations across five districts of Kashmir valley were observed for cultural and morphological variation and identification of anastomosis group causing sheath blight of rice in Kashmir.

Pathogenicity test

Pathogenicity test of *R. solani* was tested by artificial inoculation. Pathogenicity test of the seven isolates of *R.*

solani obtained from different districts of Uttar Pradesh was tested. All the tested isolates were pathogenic to potato cultivar (Kufri Bahar). The data pertaining to disease incidence and disease severity are presented in the (Table-3). Among seven isolates, RS-5 showed high disease incidence and disease severity (93.10 and 44.68% respectively) followed by RS-3 (92.30 and 42.46% respectively) while RS-13 showed least virulence and recorded minimum disease incidence and severity (82.14 and 33.53% respectively) followed by RS-7 (85.18 and 35.72% respectively) hence, the most virulent isolate (RS-5) was selected for the further studies. Similar finding was observed by earlier workers Yang *et al*., (2014) [26] pathogenicity test under glasshouse condition revealed that all binucleate *Rhizoctonia* isolates, except for the AG-1 isolates, could induce symptoms of stem canker on potato tuber. Muzhinji *et al*., (2015) [17] total 112 *Rhizoctonia solani* isolates were recovered from diseased potato plants characterized for pathogenicity. Kanetis *et al*., (2016) [10] pathogenicity and aggressiveness of the isolates was determined on 'Annabelle' potato sprouts and seedlings of a number of selected hosts, based on crop rotations followed in Cyprus. The majority of the isolates were pathogenic to potato sprouts, with disease severity values ranging from 0 to 88%.

Table 2: Survey and collection of disease sample from major potato growing districts of Uttar Pradesh.

District	Villages	Disease incidence (%)			Disease Severity (%)		
		2018-19	2019-20	Mean	2018-19	2019-20	Mean
Kannauj	Karmullapur	22	20	21	10.8	11.3	11.05
	Prempur	18	26	22	10.3	12.4	11.35
Mean 21.5 11.20							
Mainpuri	Tilyani	28	30	29	13.2	14.6	13.90
	Shivpuri	24	26	25	12.4	11.5	11.95
Mean 27.0 12.92							
Farrukhabad	Dheerpur	34	30	32	13.2	12.8	13.00
	Badanpur	22	26	24	14.5	13.6	14.05
Mean 28.0 13.52							
Etawah	Saidpur	18	20	19	8.6	9.7	9.15
	Nagla	16	14	15	9.8	7.8	8.80
Mean 17.0 8.97							
Firozabad	Budrai	36	38	37	19.2	15.4	17.30
	Hazratpur	32	40	36	16.4	17.3	16.85
Mean 36.5 17.07							
Agra	Sawai	26	30	28	15.6	16.2	15.90
	Dandaniyapur	30	28	29	14.8	15.5	15.15
Mean 28.5 15.52							
Kanpur Dehat	Bibapur	22	24	23	11.7	10.9	11.30
	Mugnisapur	18	22	20	10.8	12.6	11.70
Mean 21.5 11.50							

Table 3: Pathogenicity test of different isolates of *Rhizoctonia solani*

Inoculated isolates	No. of plants/pot	Total No. of tuber/pot	No. of infected tuber/pot	Disease incidence	Disease severity
RS-2	4	28	25	89.28	41.25
RS-3	4	26	24	92.30	42.46
RS-5	4	29	27	93.10	44.68
RS-7	4	27	23	85.18	35.72
RS-9	4	30	26	86.66	36.65
RS-12	4	25	22	88.00	39.84
RS-13	4	28	23	82.14	33.53
Uninoculated	4	26	0	00	00

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