Mycopath (2018) 16(2): 87-90 **Research Article**

Influence of abiotic factors on growth and sporulation of *Neocosmospora rubicola* associated with stem rot of potato in Punjab, Pakistan

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Abstract

Potato stem rot is wide spread disease caused fungus *Neocosmospora rubicola*. This disease has gained the status of emerging threat in most of the potato growing regions of the Pakistan. The investigations were conducted to evaluate the effect of nutrient media, temperature, hydrogen ion concentration and photoperiod on the growth condition of *N. rubicola.* The findings revealed that maximum fungal radial growth was observed at 26 ºC on PDA media having pH 7 with 16/8 light and dark period.

Keywords: Culturing conditions, fungal physiology, fungal nutrition, potato stem rot.

Introduction

Neocosmospora rubicola is a filamentous saprophytic fungus of family Nectriaceae, associated with various types of fruits, vegetables and oilseed crops rot (Lombard *et al.,* 2015). Some of the *Neocosmospora* species have been reported to cause damages in tropical and subtropical plants as well (Sandoval-Denis *et al.,* 2018). Recently, *N. rarubicola* have been reported to cause stem rot of potato in Pakistan (Riaz *et al.,* 2019). Previously it was reported as a causal agent of stem rot disease on pitaya in China and root rot of *Glycyrrhiza rulensis* in Korea (Kim *et al.,* 2017; Zheng *et al.,* 2018). The fungus produces toxins which badly affect human, animal and plant health. Dependence of microbes on temperature, pH, nutrient and site-specific ecology play significant role in determining physiology expressions (radial growth, sporulation, pattern and growth of colony) of the fungus and distribution in diversity of fungal communities (Ritchie *et al.,* 2009; Van Long *et al.,* 2017; Gordon *et al.,* 2019).

However, lack of understandings prevails on impact of environmental factors on fungal growth and its pathogenic behavior. Under controlled conditions, morphological variations in conidial size and the fungal morphological characteristics will determine suitability of a fungus to grow under specific conditions. Adaptability of a plant pathogenic fungi against climatic and plant growing conditions is referred fungal biogeography and ecosystem functions (Andrew *et al.,* 2016). Therefore, understanding the impact of interaction of a microorganism with growing conditions is essential for investigation on management studies. Sensitivity of the fungus towards different growth conditions needs to be addressed. The present study was designed to understand appropriate conditions for growth and multiplication of *N. rubicola* in which would be employed in designing pathogen virulence studies.

Materials and Methods

Effect of physiological parameters i.e. growth medium, temperature, pH and photoperiods were checked on *N. rubicola* growth rate, colony morphology and sporulation. Single spore culture medium of *N. rubicola* prepared under aseptic environment and 3 mm plug of *N. rubicola* was plated on PDA (potato dextrose agar) culture plates and incubated for 7 days (Table 1). The data on fungal growth was recorded for each test parameter at 24 hours interval. One microliter of this suspension was taken with the help of micropipette and spore count was measured using haemocytometer. Radial fungal growth in each set of treatment was recorded (cm) after incubation for 7 days.

All analytical data were statistically evaluated by using Statistix 8.1 software. One-way analysis of variance was performed by using completely randomized block design (CRD) and means of treatments were compared through least significance difference LSD at P = 0.05 level.

Results and Discussion

The relationship between growing conditions and distribution disease index is the key parameter of plant disease epidemiological investigation. The conditions primarily induce variations in pathogen to survive with environment stress conditions and degree of adaptability is referred with aggressiveness of the pathogen (Chohan *et al.,* 2015; Meena *et al.,* 2018; Gordon*et al.,* 2019).

There was variable reaction of growth rate,

colony morphology and sporulation on test growth media. The highest radial growth (8.7 cm) of *N. rubicola* was observed on PDA with maximum spore count of 5.16 × 10^6 spores mL^{-1} detectedon the generalized PDA medium, while minimum spore formation (1.46 × 10^6 spores mL^{-1}) was detected on MEA growth medium (Fig. 1), which proves *N. rubicola* a wide host range saprophytic fungus and such results previously were reported by Singh *et al.* (2019). They concluded the highest growth a fungus on generalized reflects its wide host range as it contains carbon principally for fungal nutrition purpose. These findings were also in line with Yadav *et al.* (2017) and Singh *et al.* (2019) for nutrient response on fungal growth.

In the current studies it was noted that MYA and MEA showed less mycelia growth as the lowest colony development with least number of conidia. Pradeep *et al.* (2013) have claimed that MYA and MEA mediums may contain some carbon-based compounds which other media may be missing and influences the mycelium growth and sporulation formation of *Fusarium* spp.

Temperature plays a vital role in expressing the pathogenic activity and survival of microbes; it has significant impact on the mycelium growth and spore formation of *N. rubicola. N. rubicola* can grow in between range of 14-38 ºC, same temperature range prevails in potato growing areas of the Punjab province. To find the optimum temperature for *N. rubicola*, the said temperature range was further divided in distinct limits of 14, 18, 22, 26, 30, 34, and 38 ºC. Maximum mycelia growth (8.7 cm) and spore count (5.48 × 10sporesmL^{-1}) was noticed at 26 ºC on PDA medium. Temperature below the 14 ºC significantly slow down the radial growth of *N. rubicola* and the fungus failed to sporulate even after the 7 days of inoculation. Similar trend for recoded at high temperature. However, at 38 ºC minimum mycelia growth 2.4 cm and lowest spore formation (1.61 × 10^6 spores mL^{-1}) was perceived (Fig. 2). Maximum colonial growth 8.7 cm was recorded at 26 ºC. These results are verified with the studies of Ramjegathesh and Ebenezar, (2012). Chethana (2000) stated that, most of the fungi can grow between 25 to 30 ºC. Minimum colonial growth 2.0 and 1.61 × 10^6 spores mL^{-1} was recorded at 38 ºC. Pradnyarani (2015) also studied the similar temperature range and concluded least colonial growth of *N. rubicola* induced at 35 ºC temperature.

Role of pH in fungal virulence is linked with enzymatic activities, extra cellular enzymes, endosymbiotic behavior, sporulation and mycelia growth (Madhavi *et al.,* 2012; Parveen *et al.,* 2017; Hajialilo *et al.,* 2019). Seven pH levels viz. 4, 5, 6, 7, 8 and 9 were examined by growing fungus on PDA and incubated at 26 ºC for appropriate colony growth of *N. rubicola*. In Punjab, Pakistan soil pH in general is of basic in nature and it may be as high as 8.5. Declining radial growth and spore formation was detected with the rise of pH. Maximum spore production (5.69 × 10^6 spore mL^{-1}) was noticed at pH 7.0. However, minimum (1.96 × 10^6 spores mL^{-1}) spore count was at pH4 (Fig. 3). pH level 7.0 for optimum fungal growth and sporulation was supported by Madhavi *et al.* (2012). Kumawat *et al.* (2016) suggested that maximum fungal growth can be observed in broth adjusted to pH 7 and 8 and further described importance of hydrogen ion concentration for the better fungal growth.

Role of photoperiod in fungal physiology, virulence and lipase hydrolic activity has been described (Taheri, 2019). Light intensity is also one of the significant physical factors which affect fungal growth including the establishment of reproductive structures (Fuller *et al.,* 2013; Fanelli *et al.,* 2012). Four different photoperiods were sustained at 26 ºC and highest radial growth of 8.2 cm with 5.08 × 10^6 sporesm$L^{-1}$was recorded at 16/8 hrs alternate light and dark period. The lowest growth and sporulation of 5.1 cm and 2.41 × 10^6 spores mL^{-1} was recorded at 12 h dark period (Fig. 4). Tested isolates respond differently to applied light regimes and among studied light regime highest mycelia growth 8.2 cm and 5.08 × 10^6 spores mL^{-1} was detected at 16/8 h alternate light and dark period whereas minimum was recorded at 12 h light. However, the changing physiological conditions showed a wide range of variations in colony growth pattern. The present investigations revealed that best growing conditions for growth and sporulation of the *N. rubicola* were at pH 7, 26 ºC, and 16/8 light and dark period on PDA medium. Therefore, it is assumed that finding of current studies would help in pathogen characterizations and its management.

Table 1: Effect of growth medium, temperature, pH and photoperiods on radial mycelial growth, spore germination of *N. rubicola.*

Parameters	**Growth medium**						
Medium	Potato dextrose agar **(PDA)**	Malt yeast extract agar **(MYA)**	Malt extract agar **(MEA)**	V8 juice agar **(V8)**	Fresh carrot dextrose agar **(CDA)**	Dextrose peptone malt agar **(DPMA)**	Dextrose yeast peptone agar **(DYPA)**
Temperature	14 ℃	18 ℃	22 ℃	26 ℃	30 ℃	34 ℃	38 ℃
pH	4.0	5.0	6.0	7.0	8.0	9.0	-
Photoperiods	24 h fluorescent light		24 h dark	12 h Light :12 h Dark		16 h dark : 8 h light	

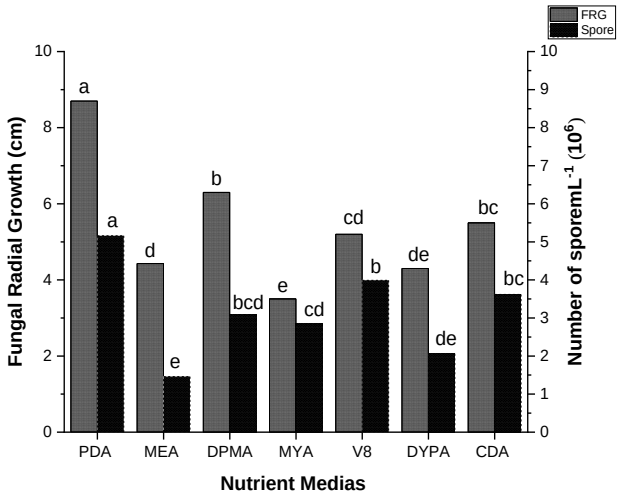

Fig. 1: Effect of nutrient medium on fungal radial growth (FRG) and spore count of *Neocosmospora rubicola*. **PDA** = Potato dextrose ager, **MYA** = Malt yeast extract, **MEA** = Malt extract agar, **V8** = V8 Juice agar, **CDA** = Fresh carrot dextrose agar, **DPMA** = Dextrose peptone malt agar, **DYPA** = Dextrose yeast peptone agar.

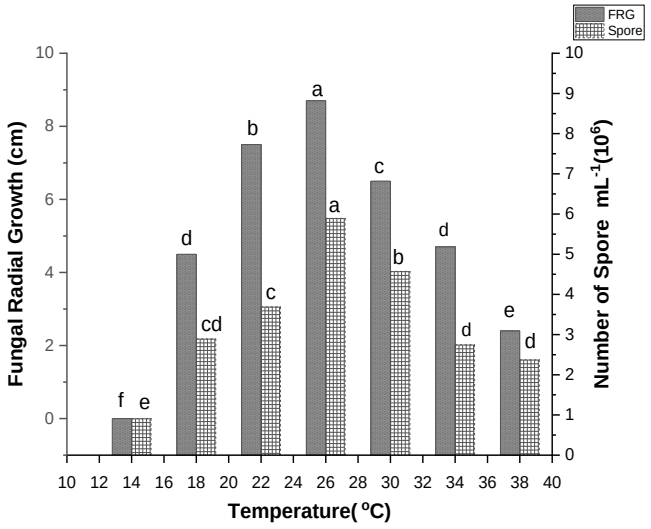

Fig. 2: Effect of temperature on fungal radial growth (FRG) and spore count of *Neocosmospora rubicola.*

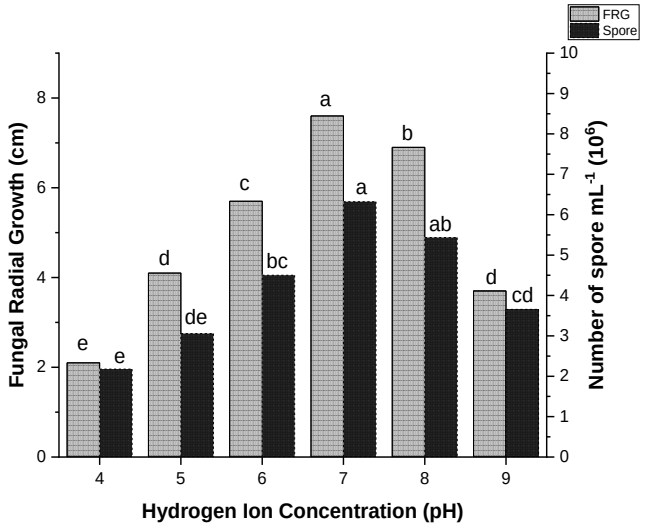

Fig. 3: Effect of various pH on fungal radial growth (FRG) and spore count of *Neocosmospora rubicola.*

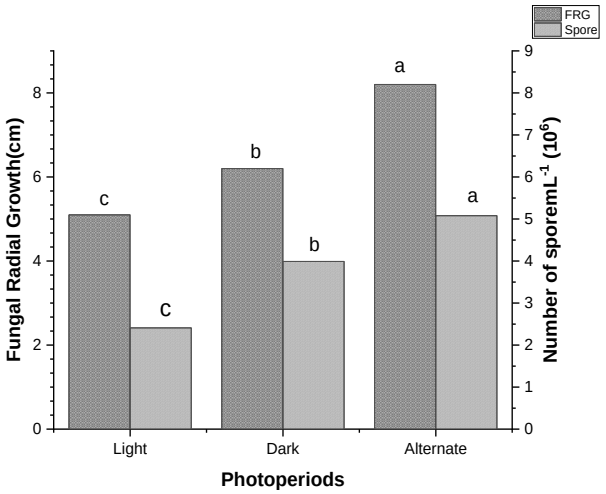

Fig. 4: Effect of photoperiod on fungal radial growth and spore count of *Neocosmospora rubicola.*

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