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"Studies on in vitro interaction of rhizospheremicroflora with *Sclerotiumrolfsii*"

[1]Poonam. P. Shete, [1*]Yuvraj. G. Kasal, [2]Pankaj. B. Deore

Corresponding author: yuvraj.20500@lpu.co.in

[1]Lovely Professional University, Phagwara (Punjab), India.
[2]Mahatma PhuleKrishiVidyapeeth, Rahuri
Plant Pathology Section, College of Agriculture, Dhule - 424 004 (Maharashtra), India.

Abstract:

Groundnut (Arachis hypogea L.) is one of the most important oilseed crops grown in Maharashtra. Stem rot disease of groundnut is caused by Sclerotiumrolfsiithe most damaging to the groundnut crop in field and causing great losses.Pathogen infected the stem and produced the dark brown lesion Form rhizosphere of groundnut stem rot infected plants Trichodermaviride was isolated. In dual culture studies it was observed that Trichodermaviride inhibited the germination and growth of all the seven isolates of Sclerotiumrolfsii. Among all the seven isolates of Sclerotiumrolfsii, hundred per cent inhibition of Sclerotiumrolfsii were recorded by Trichodermaviride. Means Trichodermaviridewas effective against all the seven isolates of Sclerotiumrolfsii.

Keywords:*rhizosphere, lesion,Sclerotiumrolfsii, growth.*

Introduction:

Groundnut is believed to be the native of Brazil. The plant was introduced by Portuguese into Africa from where it was introduced into North America. It was introduced into India during the first half of the sixteenth century from one of the Pacific Islands of China, where it was introduced earlier from either Central America or South America (Jaisani, 2009).

Leading groundnut producing countries of the world are India, China, Nigeria, Senegal, *Sudan*, Burma and USA which occupied 18.9 million ha area with 17.8 million tons production of groundnut in the world, these countries account for 69% of the area and 70% of the production. The China is the highest groundnut producer in world followed by India.

In India, groundnut is grown on 58.56 lakh hectares with production of about 82.64 lakh tons. The 58% of area and 82% of production are concentrated in Gujarat, Andhra Pradesh, Tamil Nadu and Karnataka. Andhra Pradesh, Tamil Nadu, Karnataka and Orissa have irrigated groundnut of about 6% of the total groundnut area in India (Bharati, 2010). The Gujarat is the highest groundnut producer state in India and the highest productivity is recorded in Tamil Nadu (1.64 tonnes/ha).

The total area in Maharashtra under groundnut is 2.38 lakh hectares with total production of 2.57 lakh tonnes and productivity is 1082kg/ha. Summer groundnut area in Maharashtra is

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0.824 lakh ha with production 11.96 lakh tonnes and productivity 1451 kg/ha (Anonymous, 2011-12).

Groundnut (*Arachis hypogea* L.) is a major edible oil seed crop of tropical and subtropical region of the world. The seeds, vines and dry fodder are excellent nutrient for cattle and *Rhizobium* bacterial root nodule provides nitrogen status of the soil. It has an outstanding nutritive value with 40-50% oil, 25-30% protein, on equal weight basis, groundnut contains more protein than meat and about two half times more than eggs and 18% carbohydrates in addition to minerals *i.e.,* calcium, magnesium, iron and also vitamins like B_1, B_2, E and niacin. Groundnut cake is used as cattle and poultry feed. It is also good organic manure because of its high nitrogen content (7.0-8.0%) and other nutrients. Groundnut being a legume crop fixes a significant amount of nitrogen and improves the fertility status of the soil. It is high in calories. i.e., 5.6 calories per nut (calorific value of 567 per gram), its food value is: starch (11.55%), soluble sugar (4.5%) and moisture (6%).

Less productivity in groundnut is due to many production constraints. Among these, biotic factors mainly diseases playsimportant role in limiting the yield of groundnut. This crop can be attacked by number of fungal, bacterium and viral diseases. The literature reveals that the yield losses caused by major fungal diseases like stem rot, root rot, collar rot and pod rot, singly or in combination as high as 15-70% during both *kharif*and *rabi–summer* seasons (Ghewande*et al.,* 1983, Subrahmanyam*et al.,* 1984).

*Sclerotiumrolfsii*Sacc.is widely distributed in tropics, subtropics and also in warmer parts of temperate zone of the world. In India, it is wide spread in almost all the states and causing economic losses in many crops. The numerous reports from tropical and subtropical areas of the world, joint with the large number of hosts attacked by it indicate that, economic losses are substantial every year due to infection of *Sclerotiumrolfsii*(Aycock, 1966).

*Sclerotiumrolfsii*Sacc.is a soil inhabitant, non-target, polyphagous and an ubiquitous facultative parasite. It has wide host range infecting cultivated crops *viz.,* potato, groundnut, soybean, sunflower, tomato, cotton, Lucerne, wheat and onion *etc.* It is documented that, fungus infects more than 500 plant species (Rupe, 1999).

Among the soil borne diseases, stem rot caused by *Sclerotiumrolfsii*is gaining a serious status. This disease also referred as *Sclerotium*blight, *Sclerotium* wilt, Southern blight, Southern stem rot and white mold. This fungus is distributed throughout the world and is particularly prevalent in warmer climate and significant yield losses can be seen in monoculture or short rotation with other crops which are susceptible to this pathogen (Aken and Dashiell, 1991).Among the crops *viz.,* soybean, peanut, sugar beet, pepper, tomato and potato suffer maximum losses whereas sorghum, wheat, rice, lentil, betel vine, alfalfa, cotton, sugarcane, tobacco, sunflower, chrysanthemum, gladiolus and other ornamental species suffer minor damage (Ansari, 2005). Garren (1959) has estimated the losses due to *Sclerotiumrolfsii* is to the extent of 10 to 20 million dollars annually in Southern USA

The present literature revealed that less work has been done on *in vitro* interaction of rhizospheremicroflora associated with groundnut.

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Materials and Methods:

Collection of rhizospheremicroflora samples:

The rhizospheremicroflora samples were collected by gently uprooting the stem rot infected groundnut plants using sterile shovel. The plants were shaken to remove unwanted soil particles. The soil particles adhered the roots were transferred to sterile polyethylene bags. The samples were carried aseptically to the laboratory and the rhizospheremicroflora associated with groundnut stem rot infected plants was isolated subsequently within 24 hr of sample collection.

Isolation of bioagents:

All the collected rhizosphere soil samples were pooled and representative sample was drawn. The bioagents associated with stem rot infected groundnut plants were isolated from the representative sample by following the serial dilution plate technique as detailed below.

The 10 g of representative rhizosphere soil was transferred aseptically into 250 ml conical flask, containing90 ml of sterile distilled water and the contents were mixed properly by shaking for five minutes. Then 10 ml of aliquot was drawn and transferred to 90 ml water blank (containing sterile distilled water). The suspension was shaken for one minute, before it was further diluted. Further, dilution of 10^{-2} and 10^{-4} were obtained and used for isolation of fungal bioagents.

The 20 ml of molten (40^0C) *Trichoderma specific medium* (Elad and Chet, 1983) was poured in series of Petri plates. One ml of suspension from respective dilution was transferred aseptically into a petriplates containing the medium separately. The plates were rotated manually for uniform distribution of the suspension in medium and allowed to solidify. The plates were incubated at 28 ± 2^0C for seven days for developments of fungal colonies. The colonies with characteristic growth of *Trichoderma*spp. were observed under the microscope and growth from such colonies was sub cultured on agar slants. The growth was further purified by hyphal tip culture. Thus obtained isolates of the bioagents were compared with the original description of the bioagents as given by Rifai (1969).

Interaction of bioagents with *Sclerotiumrolfsii*:

The antagonistic organisms *i.e. Trichodermaviride*was obtained and evaluated against *S. rolfsii in vitro* by dual culture technique.

For this experiment *T. viride*and *S. rolfsii*were cultured on PDA medium in petriplate separately. About 20ml of potato dextrose agar was poured into sterile petriplate and allowed to cool. A sclerotial body from actively growing culture of *S. rolfsii*was placed on one side of the petriplate and five mm disc of *T. viride* was seeded in opposite side in the same petriplate. And the same set is replicated thrice along with appropriate control (*S. rolfsii*alone). The plates were incubated at room temperature (28±2^0C) for five days.

The colony diameter of *S. rolfsii*in dual culture plate and *S. rolfsii*isolates alone inoculated plates were recorded to work out the per cent inhibition of growth of *S. rolfsii*. By *T. viride*.

The percent inhibition of mycelial growth over control was calculated by following equation given by Vincent (1927).

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$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition of *S. rolfsii*mycelium.

C = Growth of *S. rolfsii mycelium* in control on 5th day.

T = Growth of *S. rolfsii*mycelium in treatment on 5th day.

Results and Discussion:

Collection, isolation and identification of bioagents from the rhizospheremicroflora of groundnut infected with *S. rolfsii*:-

The bioagents from rhizospheremicroflora associated with groundnut stem rot infected plants were isolated subsequently within 24 hrs on *Trichoderma specific medium.* The obtained isolates of the *Trichoderma* were compared with the original description of the bioagents as given by Rifai (1969).

From the rhizospheremicroflora of stem rot infected groundnut plants *Trichodermaviride* was isolated. The colonies of *T. viride* were dark green in colour. The conidiophores were compact and mycelium was septate and hyaline. The phailides were 2 – 3, curved and narrow at base. The phialospore were globose and pale green in colour.

***In vitro* studies on interaction of *Trichodermaviride*with *S. rolfsii*isolates:**

The results presented in Table showed that the *Trichodermaviride*inhibited the germination and growth of all the isolates of *Sclerotiumrolfsii.*

Among all the seven isolates of *Sclerotiumrolfsii*recorded hundred per cent inhibition by *Trichodermaviride*.

Table.*In vitro* studies on interaction of *T. viride*with *S. rolfsii*isolates

Isolate	Diameter of *S. rolfsii* (mm)	Diameter of *S. rolfsii*in control (mm)	Per cent inhibition of *S. rolfsii*
Sr1	0.00	90	100
Sr2	0.00	90	100
Sr3	0.00	90	100
Sr4	0.00	90	100

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Sr5	0.00	90	100
Sr6	0.00	90	100
Sr7	0.00	90	100

Note : For each isolate separate untreated control (*S. rolfsii*alone) was run parallel with dual culture. Therefore, analysis is not done.

***In vitro* studies on interaction of rhizospheremicroflora with *Sclerotiumrolfsii*:**

Form rhizosphere of groundnut stem rot infected plants *Trichodermaviride* was isolated. In dual culture studies it is observed that *Trichodermaviride* inhibited the germination and growth of all the seven isolates of *Sclerotiumrolfsii.* Among all the seven isolates of*Sclerotiumrolfsii*recorded hundred per cent inhibition by *Trichodermaviride*. Means *Trichodermaviride*was effective against all the seven isolates of *Sclerotiumrolfsii.*

Similar observations were made by earlier workers while working on collar rot and root rot diseases of crop plants caused by *S. rolfsii*(Charmswarg and Sangkaha, 1988; Iqbal*et al*., 1995; Arora, 1999; Prasad *et al*., 1999 and Thiribhuvanamala*et al*., 1999).

Conclusion:

Form rhizosphere of groundnut stem rot infected plants *Trichodermaviride* was isolated. In dual culture studies it is observed that *Trichodermaviride* inhibited the germination and growth of all the seven isolates of *Sclerotiumrolfsii.* Among all the seven isolates of*Sclerotiumrolfsii*recorded cent per cent inhibition by *Trichodermaviride*. *Trichodermaviride*was effective against all the seven isolates of *Sclerotiumrolfsii.*

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