Damping-off in tobacco seedbeds caused by Rhizoctonia solani and Pythium ultimum'

S.K. Gayed[2], *D.J.S. Barr*[3], *L.K. Weresub*[3]

Pre-emergence damping-off of flue-cured tobacca (***Nicotiana tabacum*** L.) is negligible in steam sterilized seedbeds due to the lack of damping-off organisms either on or in tobacco seed produced in Ontario. Post-emergence damping-off was differentiated into: a) seedling rot initiated early where infection starts on the young leaves in touch with and spreading over the organic soil "muck", b) typical damping-off resulting from the infection of the stem of erect seedlings either directly from the soil or indirectly from already infected leaves. ***Pythium*** ultimum Trow is reported for the first time in Canada as a causal organism of damping-off in tobacco seedbeds. It caused infection at both stages without necessarily penetrating the roots, and was involved in 10% of the seedling rot cases and 25% of those of typical damping-off. However, ***Rhizoctonia solani*** Kuhn was more frequently implicated since it caused 90% of seedling rot cases and 75% of those manifesting typical damping-off symptoms at the later stage. The lethal temperature for ***R. solani*** was 60°C and for *P.* ultimum 50°C. well below the temperature reached during seedbed steam sterilization, hence the probability that seedbeds are reinfested by these organisms. Isolates of each organism varied considerably in growth, cultural characteristics, and virulence. Virulence of all isolates are partially or totally lost during 15 months in culture at 5-10°C. One isolate of ***R. solani*** produced basidia on water agar and was identified as ***Thanatephorus cucumeris*** (Frank) Donk.

Can. Plant Dis. Surv. 58:1, 15-19. 1978

La fonte des semis du tabac jaune (***Nicotiana tabacum*** L.) en prelevee est negligeable dans les couches sterilisees a la vapeur. du fait que les semences produites en Ontario sont quasi-exemptes des pathogenes responsables. En post-levee, la maladie se presente sous deux formes, une pourriture precoce des plantules qui se manifeste par l'infection des jeunes feuilles en contact avec le sol organique et une fonte typique provoquee par l'infection de la tige des plantules dressees, directement a partir du sol ou indirectement a partir des feuilles déjà infectees. On signale pour la premiere fois au Canada la presence de ***Pythium ultimum*** Trow comme agent responsable de la fonte des semis du tabac. Cet organisme a pu infecter les deux stades de croissance sans necessairement penetrer dans les racines et a cause 10% des cas de pourriture des plantules et 25% de ceux de la fonte typique, mais ***Rhizoctonia solani*** Kuhn s'est révélé plus virulent, provoquant 90% des cas de pourriture et 75% de ceux de fonte au stade de developpement plus avancé. Les temperatures mortelles de ***R. solani*** et de ***P.*** ultimum etaient de 60 et 50°C respectivement, soit bien en deçà des temperatures de stérilisation des couches a la vapeur, d'ou la probabilite que ces pathogenes reinfestent les couches. La croissance, les caracteristiques culturales et la virulence d'isolats de chaque organisme ont considerablement varie. On a observe une perte partielle ou totale de virulence de tous les isolats apres 15 mois de culture a 5-10°C. Un isolat de ***R. solani*** qui a produit des basides sur gélose aqueuse a ete identifie comme etant ***Thanatephorus cucumeris*** (Frank) Donk.

Introduction

Damping-off of flue-cured tobacco (***Nicotiana tabacum*** L.) seedlings in the greenhouse is a common disease in Canada. Despite the use of steam in seedbed sterilization or the applications of chemical sterilants, losses of tobacco seedlings in Ontario were estimated at 3-10% (5). Previous reports attributed damping-off of tobacco seedlings to ***Rhizoctonia solani*** Kuhn and ***Pythium*** spp. (5) including ***P. debaryanum*** Hesse ***(3).***

This communication establishes the involvement of another ***Pythium*** species in this disease, describes in some detail the different disease symptoms, and reports on the relative occurrence of these organisms in relation to tobacco seed and seedlings. It also reports on the heat tolerance of both organisms and the possible variation among their isolates.

Materials and Methods

In pre-emergence damping-off trials, 100 tobacco seeds produced in Ontario of cultivars Delhi 34 and White Mammoth were placed in a petri dish on moist autoclaved filter paper and on moist autoclaved muck (mixture of partially decomposed plant material, sand, silt, and clay). After incubation at room temperature for 12 days, the germinated seeds were counted. The trial was repeated twice.

The presence of fungi known to cause pre-emergence damping-off was determined by plating 100 whole or crushed Canadian seeds of flue-cured tobacco cv Virginia 115, Hicks Broadleaf, White Mammoth, and a breeding line with a germination capacity of only 44%

---

[1] ***Contribution No. 127 Research Station, Agriculture Canada, Delhi, Ont. N45 2W9.***

[2] ***Research Station, Agriculture Canada, Delhi, Ontario.***

[3] ***Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario. K I A OC6***

Accepted forpublication January 20, 1978

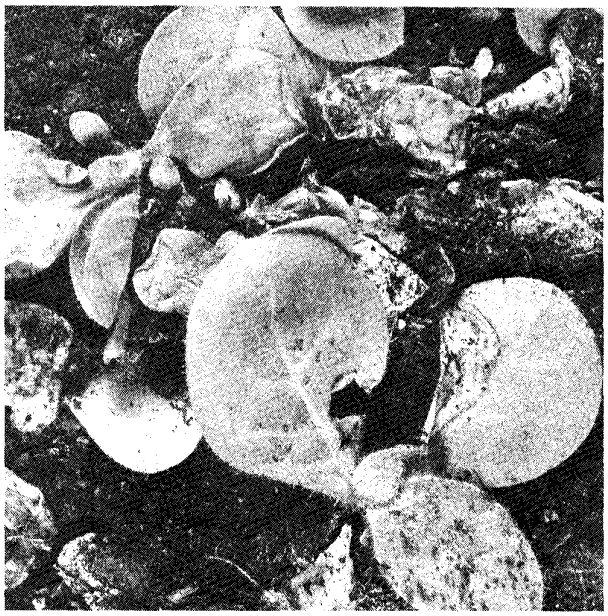

Fig. 1. Seedling rot of tobacco caused by R. solani attacking the leaves spreading on muck in a seedbed in the greenhouse.

on PDA and water agar and incubated at 25°C for 10 days. Samples of fungal growth from the seeds, seed fragments, and the sprouting seeds were examined daily. Whole seeds which failed to germinate on agar were crushed on glass slides, stained with cotton blue in lactophenol, and examined for the presence of ***Pythium,*** or ***Rhizoctonia.***

In the case of seedlings showing post-emergence damping-off, diseased and bordering healthy tissue were excised, washed with water to rid them of adhering muck particles, dipped in a 0.5% calcium hypochlorite suspension for 5 minutes for surface sterilization, and rinsed in sterile water. Fragments of this tissue were transferred to PDA, and to corn meal agar (CMA) prepared with 100 ppm pimaricin, 50 ppm polymixin B and 50 ppm penicillin G (4). Tissue fragments were stained with cotton blue in lactophenol and examined for ***Pythium*** and ***Rhizoctonia. R. solani*** could be readily identified by its coarse, broad, septate and frequently brown mycelium as compared with the fine nonseptate colorless mycelium of ***Pythium*** which carried zoosporangia and/or sexual reproductive bodies.

Soil inoculation technique was used to compare the virulence of the different isolates. R. ***solani*** was grown on 40 ml of potato dextrose broth, and ***Pythium*** on corn meal broth in 250-ml Erlenmyer flasks. After 10 days incubation at 25°C, mycelial mats were filtered off, washed, blended in 200 ml water and mixed with 500 g steam-sterilized muck. Ten 3-week old seedlings of tobacco cv Delhi 34 were transplanted into two 4-inch pots of the inoculated muck. Seedlings transplanted into steamed non-inoculated muck served as check. All seedlings were kept under moist conditions for 2 weeks during which the seedlings were observed for damage.

For the determination of the lethal temperature for ***P. ultimum,*** cultures grown on 2.4% V-8 juice agar in test tubes for 3-5 weeks and rich in oospores, were flooded with water and placed in a water bath set at various temperatures. For the determination of the lethal temperature of ***R. solani,*** cultures were grown on PDA and immature, freshly mature, and air dried sclerotial pads were similarly immersed in water in test tubes placed in a water bath. The viability of the cultures was tested by plating on PDA.

Results

Pre-emergence damping-off:

The most common fungus isolated from flue-cured tobacco seed was ***Alternaria alternata*** (Fr.) Keissler. Several species of ***Aspergillus*** and ***Penicillium*** were also present but were less abundant. Of significance to our study was the absence of ***Rhizoctonia*** and ***Pythium,*** for neither was isolated or observed in the crushed seed by direct microscopic examination. Moreover, in the 3 trials on Delhi 34 and White Mammoth the average percent germination on sterilized filter paper in petri dishes was 85, and 75; on plain agar 84 and 75; and on steam-sterilized muck 84, and 77, respectively. This indicates that pre-emergence damping-off in steamed muck is of no significance.

Post-emergence damping-off:

Damping-off usually starts to show in steam-sterilized tobacco seedbeds about 3 weeks after seeding. At this stage, the seedlings have 3-4 leaves, including the cotyledonary leaves, which spread out on the muck for up to 15 mm. During this period and for one or two weeks later when each seedling spreads out to 25-30 mm, the leaves remain in direct contact with the muck. Stem infection at soil level by damping-off organisms occasionally takes place but leaf infection is very common at this stage. Water soaked spots start to show on the leaves and gradually extend to the stem and other leaves including the growing point (Fig. 1). Diseased areas on the seedbed are in the form of circular or irregular patches of poorly-growing, yellowish, or macerated seedlings. This type of infection that starts on the spreading leaves, can hardly be considered as typical "damping-off", and "seedling rot" is proposed as a more accurate term. As the seedlings grow, typical damping-off symptoms appear, infection becomes more restricted to the base of the stem at or below soil level. Infection of the stem may take place directly from the soil, or indirectly through infection from infected leaves. Infected leaves at the base of the stem gradually shrivel and the growing mycelium extends into the stem, causing damping-off (Fig. 2). The lesion formed on the stem varies in color from brown to black, and a total separation of the top may result with a slight pull.

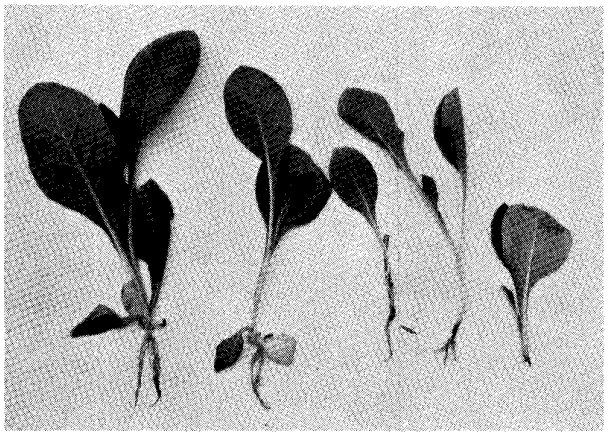

Fig. 2. From left to right, stages of leaf infection by *R. solani* leading to infection of the basal part of the stem and the separation of root from shoot.

Survey of seedling rot and damping-off organisms:
Samples of diseased tobacco seedlings mainly from greenhouses in the flue-cured tobacco area in Ontario were collected between 1967-77, including a large-scale survey carried out between 1969-71 covering 130 problem farms. Microscopic examination of diseased tissue and isolations on culture media indicated that ***Rhizoctonia*** was far more common than ***Pythium*** in young tobacco seedlings suffering from seedling rot. Of 150 samples of diseased tissue examined, ***Rhizoctonia*** was identified by direct microscopic examination and was isolated on PDA in 135 samples and ***Pythium*** only in 15 samples. The ratio of ***Rhizoctonia*** to ***Pythium*** at this early stage of seedling development was 9:1.

Later when typical damping-off symptoms were manifested on the elongated tobacco seedlings the incidence of ***Pythium*** increased slightly. Out of 163 samples, ***Pythium*** was microscopically detected and isolated from 40 samples and the remainder were caused by ***Rhizoctonia,*** thus the ratio of ***Rhizoctonia*** to ***Pythium*** was 3:1.

The causal organisms:
1. ***Pythium:*** For full identification of ***Pythium,*** 20 isolates were examined on water agar, CMA, 2.4% V-8 juice agar medium and on hemp seed in water after 7-10 days' incubation at various temperatures. Nine isolates produced oospores and were definitely identified as ***P. ultimum*** Trow. The remaining isolates were otherwise morphologically identical to ***P. ultimum,*** their response to temperature was similar, however they could not be fully identified because of the absence of the sexual state.

Considerable variation in the growth rate in culture was noted among the isolates of ***Pythium*** including those identified as ***P. ultimum.*** The average growth on PDA over a 24 hr period at 25°C varied from 29-69 mm. The growth rate was generally higher at 25 and 30°C than at 20 and 35°C with most isolates growing more rapidly at 30 than at 25°C. The optimum temperature for ***P. ultimum*** is therefore between 25-30°C and is in agreement with Lucas (6) who reported 28°C as optimum temperature for ***P. ultimum.***

No correlation was found between the rate of growth in culture and virulence of the isolates. One of the most virulent isolates grew poorly on PDA and CMA.

P. ultimum survived 46°C for 45 min. but not for 90 min., survived 50°C for only 3 min. and for less than 1 minute at temperatures higher than 50°C.

After incubation for 15 months at 5-10°C, having been subcultured three times, isolates of ***P. ultimum*** partially or totally lost their capacity to infect tobacco seedlings, although their viability and cultural characteristics were not noticeably changed.

2. Rhizoctonia: Only one isolate R1-71, of six isolates of ***Rhizoctonia,*** when transferred to water agar produced a few fertile basidia which were adequate for its determination to ***Thanatephorus cucumeris*** (Frank) Donk. The non-sporulating isolates, although highly variable in color and morphology including the size, abundance and distribution of sclerotia (Fig. 3) all demonstrated the microscopic characteristics outlined by Parmeter and Whitney (9) for ***R. solani*** Kuhn.

Isolates that caused seedling rot early in the season could not be differentiated by their culture characteristics from those that caused typical damping-off symptoms at a later stage of seedling growth. There was no correlation between the abundance or size of sclerotia or rate of mycelial spread, and virulence to tobacco seedlings. This virulence was substantially reduced or entirely lost after storage for 15 months under conditions described above for ***P. ultimum.*** The viability and culture characteristics of ***R. solani*** isolates were not noticeably changed.

R. solani grew less rapidly than ***P. ultimum,*** but still vigorously. Starting with 5 mm mycelial inoculum on PDA, the fungal growth after 24 hr at 25°C extended to 10-21 mm. Similar to ***P. ultimum,*** isolates of ***R. solani*** grew most rapidly at 25 and 30°C, many of them at 30°C. This again is in agreement with other authors including Lucas (6) who reported 28°C as optimum for ***R. solani*** causing sore-shin of tobacco. The lethal temperature for moistened sclerotia of ***R. solani*** was one minute at 60°C.

Discussion

Growers of flue-cured tobacco in Ontario prepare their seedbeds by spreading over the sandy soil a 5-cm layer of muck consisting mainly of decayed or decomposed plant material mixed with clay and sand. In order to sterilize the seedbed (7) steam is used to raise the soil temperature to 82°C at the depth of 15 cm for 30 minutes to kill disease propagules and weeds. Tobacco seeds are usually sown approximately a week after steam-sterilization of the bed.

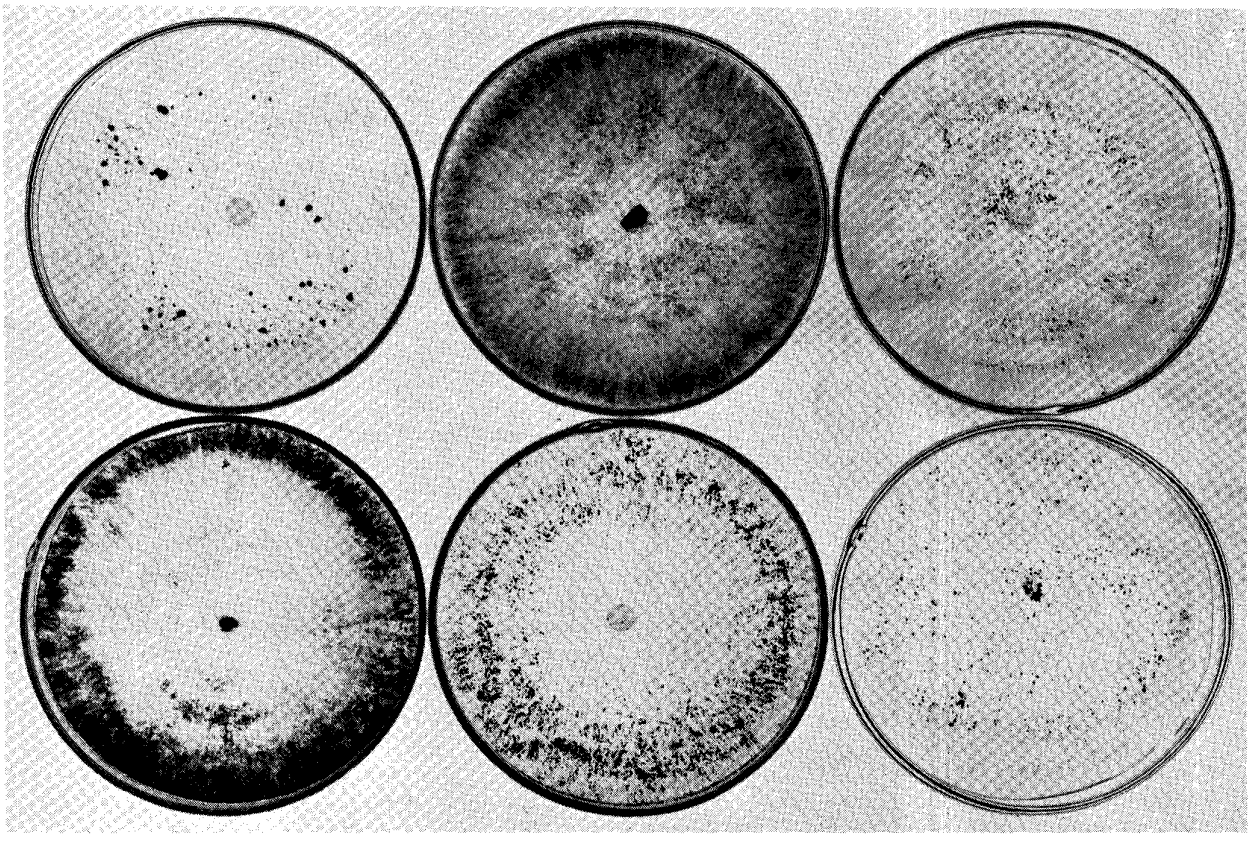

Fig. 3. Different isolates of *R. solani* on PDA showing variation in the size and the distributionof sclerotia.

In our in vitro studies exposure of less than a minute to 60°C was sufficient to inactivate not only the oospore-bearing cultures of P. *ultimum* but also the sclerotioid pads of R. *solani*. Other researchers (10) have found that sclerotia of *Rhizoctonia* are more resistant to wet heat than they were in our tests. However, in all cases reported the lethal temperature did not exceed 71°C, well below the temperature reached in steaming tobacco seedbeds. It is certain that if seedbeds are steamed according to the recommended procedures, damping-off organisms cannot survive.

Neither pathogen was among the mycoflora of tobacco seed examined, in spite of the fact that R. *solani* has been isolated in Canada from seeds of many other plants including field mustard, lettuce, flax, peas, and radish (3). Thus we have two important factors contributing to the insignificance of pre-emergence damping-off in steam-sterilized tobacco seedbeds, namely, clean seed and the low level of damping-off pathogens in the bed. Add to this the practice of tobacco growers seeding their seedbeds with soaked or sprouting seeds which reduces the period for emergence, and the advantage of the epigeal germination of tobacco seed, since evidence indicates that hypogeal germination increases risks of pre-emergence damping-off (2). Under these highly favorable circumstances, the escape of tobacco from pre-emergence damping-off in Ontario would seem to be predictable.

Post-emergence damping-off can be a serious problem in tobacco seedbeds. Within 4 weeks after seeding, disease symptoms appear on seedbed seedlings in characteristic circular or irregular patches indicating foci of re-infestation of the seedbed. It may be that the sterility of the muck itself contributes to efficient re-infestation by damping-off organisms. Baker (1) reported that the more nearly sterile the soil, the more rapid the spread of R. *solani*. He recommended soil decontamination rather than sterilization and suggested the use of aerated steam and reduced temperature that would allow the survival of antagonistic saprophytic soil microorganisms. Soil is a highly complex environment for establishing this kind of balance (8). Moreover, *Thielaviopsis basicola* (Berk & Br) Ferr. is a common pathogen in the tobacco seedbed and is controlled by steaming at 82°C as recommended by Ontario Ministry of Agriculture and Food (7).

Although previous reports show that R. *solani* is capable of infecting leaves of plants such as poinsettia (12) and China aster (13), apparently this is the first report recording the infection of leaves of tobacco seedlings with R. *solani* and P. *ultimum* directly from the soil.

Ecological studies are needed to explain why ***R. solani*** is more dominant that ***P. ultimum*** in causing damping-off in tobacco seedbeds. The relative increase in damping-off cases caused by ***P. ultimum*** from 10%to 25% in the later stages of seedling growth can be related to the possible increase in soil moisture in the bed under the leaf canopies of the dense and fast growing tobacco seedlings. This increase in moisture satisfies the hydrophytic needs of ***Pythium.***

A detailed study on R. ***solani*** isolates was necessary since the name ***R. solani*** (9) may still be in use for a complex of species. Although isolate R1-71 conformed in its mycelial-sclerotiate stage to ***R. solani,*** it could be identified as T. ***cucumeris*** but we have not dared to refer all the isolates to this species. There is, as yet, no certainty that all isolates of the still broadly circumscribed R. ***solani*** necessarily belong to T. ***cucumeris.***

The determination of ***P. ultimum*** is noteworthy. Although this species has already been implicated in the damping-off disease of many cultivated plants in Canada (3) and in tobacco in the U.S.A. (6), this is the first report of its activity in Canada on tobacco seedlings.

In respect to the site of infection, Baker (2) and others distinguish between the mode of infection of ***Pythium*** and ***Rhizoctonia,*** stressing that ***Pythium*** species generally infect root tips or root hairs and advance upward through the plants whereas ***Rhizoctonia*** causes stem decay at soil level and advances downwards. Observations made in this study and a similar observation reported by Lucas (6) indicated that ***P. ultimum*** is also capable of infecting the stem at the crown without any infection of the root.

Acknowledgements

The technical assistance of D.A. Brown and M. Kaufert is gratefully acknowledged. Thanks are also due to M.C. Watson and N. Sheidow, Ontario Ministry of Agriculture and Food, for providing most of the samples of diseased tobacco seedlings, and to O. Vaartaja for his interest in the isolates of P. ultimum and his contribution in the indentification of some of them.

Literature cited

1. Baker. K.F. 1962. Principles of heat treatment of soil and planting material. J. Australian Inst. Agr. Sci. 28: 118-126.
2. Baker. K.F. 1970. Types of ***Rhizoctonia*** diseases and their occurrence. pp. 125-133 in ***Rhizoctonia solani,*** Biology and Pathology. Ed. J.R. Parmeter, Jr. 225 pp. Univ. of Calif. Press. Berkeley, U.S.A.
3. Conners, I.L. 1967. An annotated index of plant diseases in Canada. Can. Dept. Agric. publ. 1251, 381 pp. Queen's Printer, Ottawa, Canada.
4. Eckert, J.W. and P.H. Tsao. 1962. A selective antibiotic medium for isolation of ***Phytophthora*** and ***Pythium*** from plant roots. Phytopathology. 52: 771-777.
5. Gayed, S.K. and M.C. Watson. 1975. Diseases of flue-cured tobacco in Ontario and estimates of disease losses. Can. Plant Dis. Surv. 55: 31-35.
6. Lucas, G.B. 1975. Disease of Tobacco. 621 pp. Biological Consulting Associates. Raleigh, N. Carolina. U.S.A.
7. Ontario Ministry of Agriculture and Food. 1977. Tobacco Production Recommendations. Publ. 298, 35 pp.
8. Papavizas, G.C. 1970. Colonization and growth of ***Rhizoctonia solani*** in soil. pp. 108-122 in Rhizoctonia solani, Biology and Pathology. Ed. J.R. Parmeter, Jr. 255 pp. Univ. of Calif. Press, Berkeley. U.S.A.
9. Parmeter, Jr., J.R. and R.S. Whitney. 1970. Taxonomy and Nomenclature of the Imperfect State. pp. 7-19 in ***Rhizoctonia solani,*** Biology and Pathology. Ed. J.R. Parmeter, Jr. 255 pp. Univ. of Calif. Press, Berkeley, U.S.A.
10. Sherwood, R.T. 1970. Physiology of ***Rhizoctonia solani*** pp. 69-92 in ***Rhizoctonia solani,*** Biology and Pathology. Ed. J.R. Parmeter, Jr. 225 pp. Univ. of Calif. Press, Berkeley, U.S.A.
11. Talbot, P.H.B. 1970. Taxonomy and Nomenclature of the Perfect State. pp. 20-31 in ***Rhizoctonia solani,*** Biology and Pathology, Ed. J.R. Parmeter, Jr. 225 pp. Univ. of Calif. Press, Berkeley, U.S.A.
12. Tompkins, C.M. 1959. Leaf rot of poinsettia cuttings caused by ***Rhizoctonia*** solaniand its control. Plant Dis. Reptr. 43: 1036-1037.
13. Ullstrup, A.J. 1936. Leaf blight of China Aster caused by ***Rhizoctonia solani.*** Phytopathology 26: 981-990.