J. Mycopathol. Res. ***53(1)*** *: 143-147, 2015;* (ISSN 0971-3719)

Effect of foliar spray of metalaxyl and *Trichoderma viride* on the phylloplane mycoflora of Colocasia

PEZANGULLIE CHAKRUNO* AND SUSANTA BANIK
School of Agricultural Sciences and Rural Development, Nagaland University, Dist. Dimapur, Medziphema Campus, Nagaland 797106

Received : 08.09.2014 Accepted : 10.12.2014 Published : 27.04.2015

The experiment was conducted in 2013 at the experimental field of Department of Plant Pathology, SASRD, Nagaland University, Medziphema Campus. The systemic fungicide metalaxyl (Ridomil MZ72WP) and the fungal biocontrol agent *Trichoderma viride* were sprayed at 0.3% 180 DAP (days after planting) on field grown colocasia leaves. Five leaves were sampled from each replication under the treatments randomly 1 day before treatment (DBT) and at 1, 3, 5 and 7 days after treatment (DAT). Leaf washing method was followed and Martin's Rose Bengal Agar was used for isolation of phylloplane mycoflora. Eleven fungal species *viz. Phytophthora colocasiae*, *Cladosporium herbarum*, *Penicillium citrinum*, *Aspergillus niger* isolate1, *Aspergillus niger* isolate 2, *Penicillium* mixture, *Mucor hiemalis*, *Penicillium oxalicum*, *Cladosporium macrocarpum*, *Curvularia lunata* and *Ulocladium botrytis* were observed in case of *T. viride* spray and 8 fungal species *viz. P. colocasiae*, *C. herbarum*, *P. citrinum*, *A. niger* isolate1, *A. niger* isolate 2, *Penicillium* mixture, *M. hiemalis* and *C. macrocarpum* were observed in metalaxyl spray. Foliar spray with metalaxyl resulted in significant increase in the foliar population of *C. herbarum* and *P. citrinum*, whereas that of *P. colocasiae*, *A. niger* isolate1, *A. niger* isolate2, *Penicillium* mixture, *M. hiemalis* and *C. macrocarpum* decreased significantly. In response to *T. viride* spray, *C. herbarum*, *A. niger* isolate 1, *A. niger* isolate 2, *M. hiemalis* and *C. macrocarpum* increased significantly and *P. colocasiae*, *P. citrinum*, *P. oxalicum*, *C. lunata* and *U. botrytis* decreased significantly. Seven days after treatment only 4 fungal species were observed from metalaxyl sprayed leaves compared to 8 from *T. viride* treated and 10 from water-sprayed (control) colocasia leaves.

Key words: *Phytophthora colocasiae*, metalaxyl, *Trichoderma viride*, phylloplane, colocasia, mycoflora

INTRODUCTION

Colocasia esculenta L. (Schott), also known as 'Taro' is a tropical plant belonging to the family Araceae. Colocasia are herbaceous plants up to 2 meters tall and have underground corms. The leaves are large with long petiole clasping at the base. The vegetative growth period of the crop coincides with rainy season or the monsoon period, the crop suffers heavily from Phytophthora blight disease caused by *Phytophthora colocasiae* Raciborski.

*Email : pezangulie@gmail.com

In Nagaland, colocasia occupies fifth position in terms of area under cultivation. Colocasia was cultivated in an area of 5100 ha with the total production of 48490 metric tons (Statistical Handbook of Nagaland, 2011). The crop, in Nagaland is cultivated primarily under rain-fed conditions and in jhum fields either as mono or mixed-crop with no or least amount of chemical inputs. A preliminary survey of colocasia diseases in Nagaland indicated that Phytophthora blight is the major bottleneck in its cultivation. Though there have been several effective chemical fungicides reported till date for oomycetous plant pathogens like *P. colocasiae*, the non-acceptance of chemical strategy by the Nagaland farmers warrant the researchers to devise organic strategies to ward off the disease problem.

Of several strategies available, role of microorganisms present on the leaf surface or phylloplane of colocasia remains to be explored. In recent times, the potential of phylloplane microflora in the biological control of foliar plant pathogens has become focal point of the research investigation in various crops. The term phylloplane is known to be the habitat on the leaf surface of a diverse group of microorganisms ranging from saprophytes to biotrophs (fungi, bacteria, actinomycetes etc.) whose population is dynamic being in a continuous state of interaction among them. Their interaction may have a stimulatory or inhibitory or sometimes no effect on the pathogenic microorganisms which are also present on the leaf surface at least for sometime before invading the host. The stimulatory and inhibitory effects result in corresponding increase and decrease in the incidence and severity of the disease caused by the pathogenic species. Many different microorganism spores and other propagules are likely to be randomly deposited on plant surfaces, in particular on leaf surfaces. Some of these, called transients (Forrester and Hokkanen,1994; Bonnen and Hopkins, 1997), will die or remain dormant until they are transferred to other, more suitable media, while others, may germinate under suitable conditions or start to colonize the leaf by other means, but die soon afterwards. Some of these microorganisms however, called residents (Andrews and Kinkel, 1986), may survive on the leaf surface for a longer period and together form the microflora of the phylloplane. Under suitable conditions, some may enter the leaf through stomata or wounds, or penetrate the epidermis directly. Those that do enter the leaf may grow successfully inside the leaf as endophytes, as symbionts or as parasites that cause disease. Filamentous fungi are considered transient inhabitants of leaf surfaces, being present predominantly as spores, whereas rapidly sporulating species and yeasts colonize this habitat more actively. Foliar application of fungicides on phylloplane mycoflora of Makoi (*Solanum nigrum* L.) leaf using Dithane M-45, Bavistin and Blitox and found that the leaves treated with fungicides have decreased number of fungal flora (Chauhan *et al.*, 2011), similarly foliar application of Benlate on *Solanum nigrum* L. resulted in qualitative and quantitative differences in the fungal population after treatment with the fungicide and the fungal population increased as the leaves matured (Chauhan and Jain, 2011). Phytophthora leaf blight of colocasia caused by *Phytophthora colocasiae*, an oomycetous pathogen is the most common foliar disease of colocasia in Nagaland. Metalaxyl is the systemic fungicide that is effective and recommended for oomycetous pathogens. However, its impact on the other phylloplane fungi on colocasia leaves is unknown. Hence, considering the importance of colocasia locally the present investigation was conducted.

MATERIALS AND METHODS

The research investigation was conducted in the experimental farm and laboratory of Department of Plant Pathology, School of Agricultural and Rural Development, Nagaland University, Medziphema Campus, during the year 2012-2013.

Colocasia cultivar Balsan obtained from Medziphema Town, Dimapur District of Nagaland was used for the experiment. Tubers were planted in soil at a depth of 8 cm, maintaining a distance of 60 cm row to row and 40 cm plant to plant, thus to accommodate twenty number of plants in 4 rows in each plot. The experiment was conducted following Randomized Block Design with a total of 3 treatments replicated three 3 times. The plot sizes were 2.4x2.0m^2. Planting was done on the 14th of April, 2012. Two fungicides *viz.* one chemical fungicide metalaxyl (Ridomil MZ 72WP) @ 0.3% and the other a bio-fungicide (*Trichoderma viride*, cfu-5x10^6/g) @ 0.3% were used for foliar spray. Control plots were maintained where only tap water was sprayed. At 180 DAP, 5 leaves were sampled from each plot randomly, one day before treatment (DBT) and at 1, 3, 5 and 7 days after treatment (DAT) respectively, to study the effects of foliar

spray on the phylloplane mycoflora.

Isolation of and identification of phylloplane mycoflora

All the isolation techniques carried out in this experiment were performed in an aseptic environment inside the laminar air flow chamber. Strict precautions were taken to avoid any kind of contamination. The leaf samples were collected separately in plastic bags from the field and taken to the laboratory to carry out the required isolation procedures. Five leaf samples from the basal part of the plant (Bainbridge and Dickinson, 1972) were collected separately according to the treatments in polythene bags for estimating fungal population using leaf washing method. Inside the laminar air flow chamber the leaf samples were cut into forty leaf bits of 5.0x5.0 mm^2 size. Then the leaf bits were transferred into a 250 ml sterilized conical flask containing 100 ml of sterile distilled water. The flask was thoroughly shaken for 20 minutes so that all the fungal propagules present on the leaf surface would be washed into the water. One ml of leaf washing was then transferred into a sterilized Petri-dish containing Martins agar medium (Mishra and Tewari, 1969) with the composition as follows: distilled water 1000 ml, agar agar 20 g, KH_2PO_4-1g, $MgSO_4$-0.5g, peptone-5 g, dextrose-10 g, Rose Bengal-0.033 g, and streptomycin-0.033 g . Three Martin's agar plates were inoculated and maintain as replications. The inoculated plates were sealed with Petri seal and then, they were incubated at room temperature (28±2°C). Observation was recorded on types of fungal species, colour and number of colonies of each fungal species at 2-7 days after inoculation.

The fungal colonies thus obtained were further purified and maintained in PDA (Potato Dextrose Agar: distilled water-1000 ml, peeled potato 200 g, dextrose-20 g and agar agar- 20 g) slants. The typical identifying characters of each of the phylloplane mycoflora were photographed using a digital microscope. The cultural, morphological and photographic descriptions, thus obtained were compared with description given in "Handbook of Soil Fungi" authored by A. Nagamani, I.K. Kunwar, and C. Manoharachary for identification. Further identification of all the phylloplane fungi isolated in this investigation was also made by ITCC (Indian Type Cultural Collection), Division of Plant Pathology, IARI (Indian Agricultural Research institute), New Delhi 110012, basing on their cultural and morphological characteristics.

RESULTS AND DISCUSSION

The fungi isolated from the phylloplane of colocasia are identified as given in Table 1. Data presented in Table 2 demonstrate the results of the effect of foliar spray on colocasia with metalaxyl and *Trichoderma viride*.

Effect of foliar spray of metalaxyl (Ridomil MZ 72 WP) and Trichoderma viride

The research investigation on the effect of foliar spray with metalaxyl and *Trichoderma viride* on the phylloplane mycoflora of colocasia resulted in isolation of several fungal species. The treatments under study showed changes in qualitative and quantitative composition of phylloplane mycoflora of colocasia in various dates of isolation. Altogether eleven 11 fungal species *viz. Phytophthora colocasiae*, *Cladosporium herbarum*, *Penicillium citrinum*, *Aspergillus niger* isolate1, *Aspergillus niger* isolate 2, *Penicillium* mixture, *Mucor hiemalis*, *Penicillium oxalicum*, *Cladosporium macrocarpum*,

Table 1 : Phylloplane mycoflora of colocasia and their taxonomic positions

Fungal species	Kingdom	Class	ITCC Ref. No.
Phytophthora colocasiae	Chromista	Oomycetes	9037.13
Cladosporium herbarum	Fungi	Dothideomycetes	9038.13
Penicillium citrinum	Fungi	Eurotiomycetes	9039.13
Aspergillus niger isolate 1	Fungi	Eurotiomycetes	9040.13
Aspergillus niger isolate 2	Fungi	Eurotiomycetes	9041.13
Penicillium mixture	Fungi	Eurotiomycetes	9042.13
Mucor hiemalis	Fungi	Zygomycetes	9043.13
Penicillium oxalicum	Fungi	Eurotiomycetes	9044.13
Cladosporium macrocarpum	Fungi	Dothideomycetes	9045.13
Curvularia lunata	Fungi	Euascomycetes	9046.13
Ulocladium botrytis	Fungi	Dothideomycetes	9047.13

Table 2 : Effect of foliar spray of metalaxyl and *Trichoderma viride* (@ 0.3%) on the phylloplane mycoflora of colocasia

Fungal isolates	Average number of colonies isolated in Petri plate from phylloplane of colocasia															
	1 DBT			1 DAT			3 DAT			5 DAT			7 DAT			
	Control	Ridomil	*T. viride*	Control	Ridomil	*T. viride*	Control	Ridomil	*T. viride*	Control	Ridomil	*T. viride*	Control	Ridomil	*T. viride*	Mean
Phytophthora colocasiae	0.00 (0.71)*	0.11 (0.78)	2.11 (1.62)	1.00 (1.22)	0.89 (1.18)	2.11 (1.61)	1.00 (1.22)	2.11 (1.61)	0.77 (1.13)	1.87 (1.54)	0.00 (0.71)	3.22 (1.93)	1.89 (1.54)	0.77 (1.13)	1.55 (1.43)	1.29
Cladosporium herbarum	0.33 (0.91)	7.00 (2.74)	0.44 (0.97)	1.22 (1.31)	1.22 (1.31)	1.89 (1.54)	1.44 (1.39)	6.33 (2.61)	2.55 (1.75)	5.66 (2.48)	7.00 (2.74)	5.11 (2.37)	9.77 (3.21)	16.33 (4.10)	13.89 (3.78)	5.35
Penicillium citrinum	9.09 (3.10)	0.44 (0.97)	32.88 (5.78)	3.00 (1.87)	0.33 (0.91)	2.66 (1.78)	1.33 (1.35)	0.33 (0.91)	0.00 (0.714)	2.11 (1.61)	0.88 (1.18)	1.00 (1.22)	11.00 (3.39)	4.66 (2.27)	0.55 (1.03)	4.68
Aspergillus niger isolate 1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.33 (0.91)	0.00 (0.71)	0.11 (0.78)	0.66 (1.08)	1.44 (1.39)	0.00 (0.71)	0.66 (1.08)	0.00 (0.71)	0.00 (0.71)	0.55 (1.03)	0.00 (0.71)	0.44 (0.97)	0.28
Aspergillus niger isolate 2	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.11 (0.78)	0.55 (1.03)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.66 (1.08)	0.22 (0.85)	0.00 (0.71)	0.78 (1.13)	0.18
Penicillium mixture	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.22 (0.85)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.77 (1.13)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.00 (0.71)	0.22 (0.85)	0.00 (0.71)	0.00 (0.71)	0.10
Mucor hiemalis	0.44 (0.97)	0.55 (1.02)	0.00 (0.71)	0.55 (1.02)	0.00 (0.71)	0.77 (1.13)	0.33 (0.91)	0.00 (0.71)	1.66 (1.47)	0.22 (0.85)	2.00 (1.58)	1.22 (1.31)	0.77 (1.13)	1.55 (1.43)	2.33 (1.68)	0.83
Penicillium oxalicum	0.10 (0.77)	0.00 (0.71)	0.22 (0.85)	0.22 (0.85)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.00 (0.71)	0.04
Cladosporium macrocarpum	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.00 (0.71)	0.22 (0.85)	0.00 (0.71)	0.22 (0.85)	0.04
Curvularia lunata	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.11 (0.78)	0.11 (0.78)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.02
Ulocladium botrytis	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.11 (0.78)	0.00 (0.71)	0.33 (0.91)	0.11 (0.78)	0.00 (0.71)	0.11 (0.78)	0.04
Mean	0.91	0.75	3.24	0.59	0.23	0.69	0.46	1.06	0.46	1.01	0.90	1.05	2.26	2.12	1.81	

	MSE	CD (P= 0.05)
Fungus(F)		
Treatment(T)	2.84	0.816
F x T	2. 84	0.69
	2.84	2.70

*Figures in the parentheses indicate square root transformed values

Curvularia lunata and *Ulocladium botrytis* were observed in case of *Trichoderma viride* spray and 8 fungal species *viz. P. colocasiae*, *C. herbarum*, *P. citrinum*, *A. niger* isolate1, *A. niger* isolate 2, *Penicillium* mixture, *M. hiemalis* and *C. macrocarpum* were observed in metalaxyl spray in different dates of isolation. The highest average number of colony count (3.22) for *P. colocasiae* was observed in *T. viride* spray at 5 DAT, that of *C. herbarum* in Ridomil spray at 7 DAT (16.33), *P. citrinum* in *T. viride* spray at 1 DAT (32.88), *A. niger* isolate 1 in Ridomil spray at 3 DAT (1.44), *A. niger* isolate 2 in *T. viride* spray at 7 DAT (0.78), *Penicillium* mixture in Ridomil spray at 3 DAT (0.77), *M. hiemalis* in *T. viride* spray at 7 DAT (2.33), *P. oxalicum* in *T. viride* spray at 1 DBT (0.22) and in control at 1 DAT (0.22), *C. macrocarpum* in both control and *T. viride* spray at 7 DAT(0.22), *C. lunata* in control and *T. viride* spray at 3 DAT (0.11) and in control at 5 DAT (0.11), *U. botrytis* in *T. viride* spray at 5 DAT (0.33) as given in Table 2.

It was observed that the foliar spray with metalaxyl (Ridomil MZ 72WP) and *T. viride* showed significant increase and decrease in the phylloplane population respectively. Metalaxyl spray at 7 DAT resulted in yield of only four fungal species *P. colocasiae*, *C. herbarum*, *P. citrinum* and *M. hiemalis* compared to 10 fungal species in control. Out of the four fungal species, the average colony count of *P. colocasiae* and *P. citrinum* was reduced from 1.89 in control to 0.77 and 11.00 in control to 4.66 respectively. However, that of *C. herbarum* and *M. hiemalis* increased significantly. On the other hand, with *T. viride* spray, colony count of *C. herbarum*, *A. niger* isolate 1, *A. niger* isolate 2, *M. hiemalis* and *C. macrocarpum* increased significantly and *P. colocasiae*, *P. citrinum*, *P. oxalicum*, *C. lunata* and *U. botrytis* decreased significantly. Similar type of observation was reported by Mehan and Chohan (1981) on groundnut that foliar treatments with fungicides have correlation with the increase and decrease in the phylloplane mycoflora. Southwell *et al.* (1999) observed that spray on barley and wheat with mancozeb reduced the fungal population and triadimefon had only a minor effect on the fungal population. The findings of these workers fully support the present result. Of all the phyllplane mycoflora of colocasia isolated in the present study, *C. lunata* was the least abundant and *C. herbarum* was the most abundant with average colony count of 0.02 and 5.35 respectively considering all the dates of isolation from all treatments. It seems logical to point out that some fluctuation in the data were observed which might have correlation with the rainfall that was observed while carrying out this experiment. *T. viride* foliar application was done 180 DAP. But subsequent isolation of phylloplane mycoflora after foliar spray did not yield *T. viride* which indicates that *T. viride* could not survive on phylloplane. However, its metabolites or contents of dead cells released in on phylloplane could have favoured and/or prevented the growth of other mycoflora.

ACKNOWLEDGEMENT

The authors are thankful to Prof. L. Daiho, Head, Department of Plant Pathology, School of Agricultural and Rural Development, Nagaland University, Medziphema Campus, for his inspiration on the investigation, by giving creative suggestion, encouragement and providing all the available facilities throughout the investigation.

REFERENCES

Andrews, J.H. and Kinkel, L.L. 1986. Colonization dynamics: The island theory. pp. 63-76 *In*: *Microbiology of the phyllosphere*, Fokkema, N.J. and van den Heuvel, J. (Eds); Cambridge University Press, New York.

Bainbridge A and Dickinson C H 1972 Effect of fungicides on the microflora of potato leaves. *Transactions of British Mycological Society* **59:** 31-41.

Bonnen, A.M and Hopkins, C 1997. Fungicide resistance and population variation in *Verticillium fungicola*, a pathogen of the button mushroom, *Agaricus bisporus*. *Mycological Research* **101:**89-96.

Chauhan, J. and Jain, D. K. 2011. Effect of Benlate on the phylloplane mycoflora of *Solanum nigrum* L. *Journal of Plant Development Sciences*. **3**: 329-330.

Chauhan. J., Jain, D. K., Pande, P. C. and Singh, P. N. 2011. Effect of foliar application of fungicides on phylloplane fungi of Makoi leaf. *Journal of Plant Development Sciences*. **3**: 117-126.

Forrester, N.W. and Hokkanen, H.M.T. 1994. Resistance management options for conventional *Bacillus thuringiensis* and transgenic plants in Australian summer field crops. *Biocontrol Science and Technology*. **4**: 549-553.

Mehan, V. K. and Chohan, J. S. 1981. Effect of fungicides on leaf-spot pathogens and the phylloplane mycoflora of groundnut. *Transactions of the British Mycological Society*. **76** : 361-366.

Mishra R R and Tewari R P 1969 Plant height as a factor in relation to leaf surface mycoflora of *Cassia tora*. *Indian Journal of Microbiology* **9**:73-76.

Southwell, R. J., Brown, J. F. and Welsby, S. M. (1999) Microbial interactions on the phylloplane of wheat and barley after applications of mancozeb and triadimefon. *Australasian Plant Pathology*. **28** : 139-148.

Statistical Handbook of Nagaland 2011. Directorate of Economics & Statistics, Nagaland.