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Synergism and antagonism among the fungi grown in honeydew secretion on leaf surface of Nagpur mandarin

V.K. SRIVASTAVA* and R.P. THAKRE
Department of Botany, Nagpur University Campus, Nagpur 440 010

ABSTRACT: Certain insect infestation results in honeydew secretion, which governs the development of the synergistic association by altering the leaf surface in such a way that it becomes suitable for colonization by sooty mould in association with several other saprophytic fungi. This situation was created on the leaf surface of citrus by certain honeydew secreting insects, viz., citrus black fly, citrtus whitefly and citrus psylla. They played a synergistic role by providing a platform coupled with required nutrients in the form of honeydew on the citrus leaf surface for the growth of the sooty moulds, alongwith other saprophytic phylloplane fungi. Second type of synergism observed was between and among the saprophytic fungi colonized over honeydew on citrus leaf surface. Prominent sooty mould fungus was *Capnodium citri*. It absorbed the nutrients from insect honeydew from the citrus leaf surface and was a pioneer colonizer, and being a filamentous fungus added fungal biomass to it. There were further additions in the quantum of sooty mould biomass by the other black or brown colour fungi viz., *Alternaria alternata, Botryodiplodia theobromae, Cladosporium oxysporum Colletotrichum gloeosporioides, Curvularia spp., Hansfordia nebularis, Nodulosporium sp.* and *Torula allii*, and in association they formed sooty mould complex. *H. nebularis* was found to inhibit the growth of *C. oxysporum* when grown separately, but did not affect the growth of the other members of sooty mould complex. Antagonism between the fungi of sooty mould complex and other saprophytes was also observed *in vitro*. The prominent antagonist among all the mycoflora was *T. viride*. It was found antagonistic to all the members of sooty mould complex.

Key words: Antagonism, honeydew, mould complex, mycoparasitism, phyllosphere, sooty mould, *Citrus reticulata*.

Knowledge of saprophytic fungi colonizing the phyllosphere has increased considerably. Interest is mainly due to the possible harmful effects of saprophytes on crop yields, but there are several reports that common fungal leaf saprophytes antagonize plant pathogens. The saprophytic fungi colonizing the phyllosphere have the characters to synergize with each other. On the citrus leaf surface, several saprophytic fungi grow in honeydew secretion of insect in association with specific sooty mould i.e., *Capnodium citri*. Their subsequent vegetative development on the citrus leaf surface is more extensive depending on the availability of nutrients. Certain insect infestation causes damage to the citrus orchards and supply nutrients on the leaf surface in the form of honeydew secretion to the sooty mould and its associate members. Present study has been done to see the interrelationships among the fungi occurring in the honeydew on the leaf surface of cirtus.

* Central Integrated Pest Management Centre, New Secretariat Building, IInd floor, East Wing, Civil Lines, Nagpur - 440 001

MATERIALS AND METHODS

Degree of the infestation level of insects viz., citrus blackfly (*Aleurocanthus woglumi*), citrus whitefly (*Dialeurodes citri*) and citrus psylla (*Diaphorina citri*) on citrus orchards and subsequent presence of honeydew on the aerial organs viz., leaves, twigs, branches and stems were recorded during the monthwise seasonal surveys for the study of synergism between the amount of nutrition supplied by insects in the form of honeydew and sooty moulds developed on the leaf surface in association with several other fungal saprophytes. Total 45 fungi were isolated from the leaves of citrus orchards through cultural technique (Dickinson, 1971). The synergism among the fungal saprophytes grown in honeydew was studied in laboratory on culture media PDA (potato-dextrose agar). The fungi showing similar symptoms especially black or brown colour were grown simultaneously on PDA and their behaviour, growth pattern and appearance were studied.

Ten fungi showing more or less sooty mould symptoms, viz., *Alternaria alternata, Botryodiplodia* *theobromae, Cladosporium oxysporum Colletotrichum gloeosporioides, Curvularia lunata, C. tetramera, C. turberculata, Hansfordia nebularis, Nodulosporium sp.* and *Torula allii* were inoculated together on PDA and their simultaneous growth was observed. These fungi were regrouped by taking two members at each occasion and then inoculated simultaneously. Thus 45 groups, each of two fungi were formed. Synergism among these 10 fungi was also tested in glass-house condition. A thick layer of honey was coated on the ventral side of the citrus leaves and then the spore suspension of all the 10 fungi was sprayed simultaneously on the same leaves and also separately to observe their growth on the leaf surface *in vivo*. Sprayed leaves with the fungi were covered with polythene bags for 24 hours to provide high humid condition to the test fungi.

These 10 fungi were again grown with rest of the 35 fungi to see the antagonistic effect with each other by adopting dual culture technique on PDA. These fungi were : *Absidia corymbifera, Acremonium sp., Aspergillus candidus, A. flavus, A. glaucus, A. nidulans, A. niger, A. niveus, A. sulphureus, A. sydowi, A. tamarii, Cephalosporium acremonium, Chaetophoma sp., Fusarium aquaedutuum, G. moniliforme, F. oxysporum. G. semitectum, Monilia sp., Mucor hiemalis, M. microsporus, Paecilomyces variotti, Penicillium aurantiogriseum P. restrictum, P. oxalicum, Penicillium sp., P. variabile, Phytophthora palmivora, Pythium sp., Rhizoctonia sp., Rhizopus microsporus, R. stolonifer, Stemphylioma terricola, Syncephalastrum recemosum, Trichoderma viride, Trichothecuim roseum.*

T. viride being the prominent antagonist and having all or more than one mode of antagonism i.e., food competition, antibiosis and parasitism/predation, was again tested with all the fungi encountered through culture method for its antagonistic effect.

Antagonistc effect of *T. viride, in vivo* was also studied in glass house condition. Spore suspension of *T. viride* 10^{10} cfu/ml was sprayed on honeydew coated leaves of citrus. The spore suspension of the test fungi was also sprayed on the same leaves simultaneously and alone too on the same day and at the day before and after the spraying of *T. viride* spore suspension. The leaves sprayed with these fungi were covered with polythene bags for 48 hrs. other than the leaves on which sooty mould fungi and *T. viride* were sprayed on same day. The leaves sprayed with *T. viride* and sooty moulds on same day were covered for 24 hrs. only. Visual observation on the development of sooty moulds and antagonistic effect of *T. viride* was done at two days interval for one month. At the end of the month, 3 leaves from each treatment were taken and cut into small bits, then thoroughly washed in 100 ml. of water to get the spore suspension. The spore suspension was filtered with the help of muslin cloth. Then 1 ml suspension was taken in sterilized petriplates and 20 ml PDA was poured in each petriplate. Three replications of each treatment were made. Now petriplates containing spores of test fungus alongwith PDA nutrient were incubated at 25 ± 1°C in BOD incubator for 7 days. Colonies developed in each petriplate were counted.

RESULTS AND DISCUSSION

Occurrence of different fungi on leaf surface of citrus was totally depended upon the quantity of honeydew present therein. Quantity of honeydew was directly proportionate to the intensity of infestation of insects viz., citrus blackfly, citrus whitefly and citrus psylla, because these were actually responsible for secreting honeydew on the leaf surface. Young citrus orchards showed less development of sooty moulds because of less infestation of insects.

When the fungi (10nos.) were grown in the PDA poured petriplate, they exhibited typical appearance of sooty mould, and were closely associated with actual sooty mould i.e., *Capnodium citri* in field condition. When these fungi were regrouped in several combinations, most of them grew freely without affecting the growth of others except *Hansfordia nebularis* and *Nodulosporium sp.* Both the fungi were found affecting the growth of *Cladosporium oxysporum* and *Colletotrichum gloeosporioides.* Although *Nodulosporium sp.* was found overgrowing on both the fungi and restricting their growth, parasitization of hyphae of any of the fungus could not be observed.

When these test fungi were tested *in vivo*, honey coated leaves sprayed with all fungi together showed the symptom of sooty mould. The honeydew coated leaves on which these fungi were sprayed separately did not exhibit the typical sooty mould appearance except in the case of *Botryodiploida theobromae* and *Cladosporium oxysporum*. The leaves on which *Colletotrichum gloeosporioides* and *Nadulosporium sp.* were sprayed showed dirty cottony growth. *C. oxysporum* and *Torula allii* also showed typical sooty mould growth. The growth of other fungi viz., *Alternaria alternata, Curvularia spp.* and *H. nebularis* was not so luxuriant, but visible on the citrus leaf surface.

Test fungi (10) when grown with rest of the fungi to test their antogonism 13 out of them were found antagonistic to different fungi in different ways of action. *Hansfordia nebularis* and *Nodulosporium sp.*

Table 1. Details of fungi antagonizing test fungi and their mode of action

Test fungi	Fungi which antagonized test fungi due to		
	Food competition	Antibiosis/Metabolites	Parasitism
Alternaria alternata	*Rhizopus spp., Trichoderma viride*	*Aspergillus spp., Penicillium restrictum, T. viride*	*T. viride*
Botryodiplodia theobromae	*Mucor microsporus, Rhizopus spp., T. viride*	*Aspergillus spp., P. restrictum, P. oxalicum, P. oxalicum, P. aurantiogriseum, Monilia sp., T. viride*	*T. viride*
Cladosporium oxysporum	*Rhizopus spp., Nodulosporium sp.*	*Aspergillus spp., P. restrictum, T. viride*	*Hansforodia nebularis, T. viride*
Colletotrichum gloeosporiodes	*Nodulosporium sp. T. viride*	*Aspergillus spp., P. restrictum, P. oxalicum, Monilia sp., T. viride.*	*T. viride*
Curvularia lunata	*Rhizopus, T. viride*	*Aspergillus sp., P. restrictum, P. oxalicum*	*T. viride*
C. tetramera	*Rhizopus spp., T. viride*	*P. restrictum, P. oxalicum*	*T. viride*
C. tuberculata	*Rhizopus spp., T. viride*	*Aspergillus sp., P. restrictum*	*T. viride*
Hansfordia nebularis	*T. viride*	*T. viride*	*T. viride*
Nodulosporium sp.	*T. viride*	*Aspergillus spp., P. oxalicum, P. restrictum, Monilia spp*	*T. viride*
Torula allii	*M. microsporum, Rhizopus spp., T. viride*	*Aspergillus sp., P. restrictum, P. oxalicum, Monila spp., T. viride*	*T. viride*

Aspergillus spp. (*A. niger, A. flavus, A. tamarii* and *A. candidus*); *Rhizopus spp.* (*R. microsporus* and *R. stolonifer*)

Table 2. No. of colonies observed at one month after spraying of test fungi alone and with *T. viride* on citrus leaves

Test fungi	Average no. of colonies observed after one month of spraying of test fungi			
	Alone	Test fungi + *T. viride*		
		One day before	Same day	After one day
Alternaria alternata	8.7	7.3	7.7	8.7
Botryodiplodia theobromae	18.3	12.7	17.7	17.3
Cladosporium oxysporum	32.7	30.7	31.3	32.3
Colletortrichum gloeosporioides	5.7	4.7	5.3	5.3
Curvularia lunata	14.7	15.3	14.7	15.7
C. tetramera	10.7	10.7	11.3	10.7
C. tuberculata	16.7	16.7	17.3	18.3
Hansfordia nebularis	3.3	3.3	4.7	2.7
Nodulosporium sp.	6.7	5.7	6.7	6.3
Torula allii	13.3	12.3	13.7	13.3

were also found antagonistic to *C. oxysporum* and *C. gloeosporioides*, when grown with these fungi separately. Results are summarized in Table 1.

Antagonistic effect of *Trichoderma viride* shown in Table 2 revealed that its antagonistic effect on the test fungi, *in vivo* but glass house condition did not show any effect.

Most important source of extraneous nutrients is honeydew which facilitate the growth of sooty mould complex (Huges, 1976). Honeydew rarely stimulates infection in the field because the insect population, and consequently the deposition of honeydew, increase gradually which allows sufficient time for the saprophytic scavengers to remove these nutrients

(Fokkema, 1980). It was seen that primary fungal saprophytes getting nourishment form honeydew on citrus leaves were not only the limiting micro-organisms for producing sooty mould bio-mass, simultaneously some other fungi were also found to be associated with sooty moulds, although their individual appearance was unlike sooty moulds. These fungi grew freely without affecting the growth of sooty moulds. The propagules of the fungi occurred on the same leaf commenced actively little bit earlier, late or at the same time with sooty moulds and definitely added fungal bio-mass on the citrus leaf surface. In this way high concentration of propagules were accumulated on the leaves and other aerial tissues. These fungi also enjoyed their life on the aerial parts of citrus orchards getting nutrients from honeydew and remained in dormant stage there, until favorable combination of environmental conditions triggers mass germination and growth. It was observed that these fungi also played a synergistic role in enhancing the fungal bio-mass on the leaf surface in association with sooty moulds.

It is evident from the Table 1 that some saprophytic fungi played important role in suppressing the growth of other saprophytes due to their competitive saprophytic ability. Four species of *Aspergillus, Monilia spp.* and *Trichoderma viride* released their metabolites which performed antibiotic activity.

Inhibitory substance produced by *Aspergillus spp.* are known to play an important role in soil fungistatis (Johri and Singh, 1975). Such substances have been reported from culture filtrates of *A. niger, A. flavus and A. candidus* (Shukla and Dwiwedi, 1979). Among the species of Aspergillus isolated from citrus leaf surface, *A. niger, A. flavus, A. candidus* and *A. tamarii* were found to have such substances which affected the growth of most of the test fungi except *Curvularia tetramera* and *Hansfordia nebularis.*

Neither nutrient competition nor mycoparasitism was observed in the case of *Penicillium spp.* Clear antibiotic effect was also not observed. Metabolites were present, but there was no indication whether these had actually checked the growth of test fungi. Despite all above facts, *Penicillium spp.* were found affecting the growth of test fungi. *T. viride* affected the growth of fungi by both way i.e., food competition and antibiosis. Mycoparasitism has a wider meaning than hyperparasitism (Kranz, 1981). *Hansfordia nebularis* isolated from citrus leaf surface was found parasitizing *Cladosporium oxysporum*. There is report that *H. pulvinata* is antagonistic to *C. fulvum* (Peresse and Le Picard, 1980).

Certain saprophytic fungi, usually a relatively small number are mentioned in the literature as antagonists reaching plant surface naturally by aerial dissemination (Dubos and Bulit, 1980). *Trichoderma spp.* are such type of saprophytic fungi. *T. viride* was the most prominent mycoparasite which parasitised almost all the test fungi except *Curvularia tuberculata*. When it was grown with the fungi viz., *B. theobromae, C. oxysporum* and *T. allii*, it also affected the release of metabolites from these fungi. It also antagonized *Fusarium spp., Phytophthora palmivora, Pythium sp.* and *Rhizoctonia sp.*

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