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A NEW TECHNIQUE OF HARVESTING AND ISOLATION OF PLANT PATHOGENIC *FUSARIUM* FROM *FUSARIUM* AFFECTED SOIL

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ABSTRACT
Isolation of plant pathogenic *Fusarium spp.* from soil samples of wilt infected crop soils is a tedious task due to appearance of several fungal, bacterial and *actinomycetes spp,* present in such soil sample, in the isolation plates. A new technique for harvesting and isolation of plant pathogenic *fusarium directly* from soil samples was developed. Out of different fruits and vegetables slices charged with the infected soil sample, the ripen tomato fruit slices was able to harvest the *fusarium* pathogen with its fungal growth and sporulation. The harvested *fusarium,* then was easy to grown on routinely used fungal medium. Isolation of *Fusarium* directly from the soil sample, on tomato disc is a new technique of harvesting of *Fusarium* from fusarium infected soil samples and can be used by plant disease diagnostic labs for assessment of presence of *Fusarium* wilt pathogen in the soil. This is a new and cheap technique of assessment of fusarium in the soil samples rather than using a costly specialised media for the isolation of fusarium directly from soil samples.

INTRODUCTION

Fusarium is an important soil borne fungal pathogen infecting plant root system to cause wilt in several crop plants with considerable losses (Warda Jendoubi, *et al.,* 2017). The pathogen has pathogenic variability among them (Chopada *et al.,* 2014) and can be primarily control by use of fungicide and bio-agent trichoderma (Anita kumari *et al.,* 2014). Recently the wilt caused by fusarium species is controlled by chitosan (Bouthenia *et al.*, 2019) and non-pathogenic isolates of fusarium species (Suresh Patil and Subbaraman Sriram, 2020).

It is easy to isolate the *fusarium* from infected root samples rather than directly from soil samples; as the isolation from soil sample yields several other fungi, bacteria and *actinomycetes spp.* When only the soil samples are provided for the assessment of presence of fusarium in the soil samples, a need of a technique to harvest and isolate the *fusarium* pathogen was needed.

No such technique of harvesting and isolation of *fusarium* exclusively from the soil samples without any other microbial contamination is available in the literature.

As our diagnostic laboratory receives the soil samples for the detection of presence of *fusarium*, the need was felt for the technique to harvest the *fusarium* directly from soil samples. It was thought whether the *fusarium* can be harvested directly from the soil samples either on soft fruit slices, vegetable slices and on tapioca granules; and therefore these were employed in the present study with an objective to develop a technique for routine assessment of fusarium from soil samples for the diagnostic laboratories.

MATERIALS AND METHODS

The standard laboratory instruments like Laminar air flow cabinet, BOD Incubator, Desiccator, and borosil make glassware were used during the investigations. For harvesting and isolation of the *fusarium* from soil samples the following methods were employed.

Isolation of *fusarium* directly from wilt affected soil sample by dilution plating method

The soil samples from citrus orchard infected with wilt disease pathogen, supplied by the cultivator in Jalgaon district (Maharashtra state),was used for isolation of soil borne wilt causing pathogen *fusarium* by direct dilution plating method on TAPS medium (Borkar and Bhattacharjee 2014; Jayshree Bhattacharjee *et al.*, 2015). The inoculated plates were incubated at 29±1 °C in BOD incubator for 72 hours and growth of wilt causing *fusarium* was recorded.

Assessment of use of sliced fruit/vegetable for harvesting of soil borne *fusarium* directly from the soil samples

Besides causing wilt in plants, the *fusarial* pathogen also infect fruits and vegetables to cause soft rot (Avinash Ingle, 2017). Taking this into account, the use of sliced fruit/vegetables for harvesting of soil *fusarium* was thought of. For harvesting of *fusarium* pathogen directly from soil samples a specialized

technique was developed and standardized in our laboratory. For this 5 mm slice of the two fruits i.e. pear and apple and two vegetable fruits i.e. tomato and cucumber were used.

The soil suspension of infected soil sample was made by adding 50gm soil in 100ml water; allowed to settle for 15 minutes and an aliquot of 15ml from this suspension was taken in individual glass petri-plates. The sliced fruit discs were half immersed in the soil suspension in such a way that the lower portion of sliced discs were in contact with soil water suspension while upper portion of sliced discs remain out of contact of the suspension. The sliced discs were kept overnight in the soil water suspension at ambient room temperature. The next day these sliced discs were removed with the help of forceps with due care and placed on moist blotter paper facing the soil water immersed portion of sliced disc upward in petri plates. These samples plates were kept in desiccator for 72 hours for the growth of fungi harvested on these sliced discs.

Assessment of use of tapioca granules for harvesting of soil borne *fusarium* directly from the soil samples

For this, the tapioca granules (100 in number) were water-soaked for 20 minutes in distilled water. The soil sample was taken in petri plates and the water-soaked tapioca granules were placed randomly on the soil surface and pressed to insert halfway into the soil. This tapioca granule plate was kept in a water desiccator for the growth of fusarium fungi on the granules. The fungal growth appeared on these granules was examined microscopically for assessment of *fusarium* pathogen.

Assessment of harvested fungal growth on sliced fruit disc/ tapioca granules

To assess and identify the harvested fungal growth on sliced fruit disc /tapioca granules (Figure 1), the fungal growth present on sliced discs were observed microscopically and confirm as fusarium fungi on the basis of their morphological spore structure (Porter *et al.*, 2015; Summerell and Leslie, 2006). The fusarium growth from the respective fruit disc were picked up and placed on TAPS medium to obtain pure growth of the fusarium.

The experiment was repeated five times for the confirmation of harvesting of fusarium on tomato slice disc from the fusarial infected soil sample.

A- Saprophytic fungal growth on soaked tapioca granules placed in *fusarial* infected soil sample, B - Saprophytic fungal growth on dry Tapioca granules inserted halfway in infected soil sample, C - Saprophytic fungal growth on Cucumber disc. D: Saprophytic fungal growth on Pear fruit disc, E - *Fusarium* growth on Tomato disc, and F: Saprophytic fungal growth on Apple fruit disc.

Figure 1: Assessment of sliced fruit disc and tapioca granules for harvesting the fungus *fusarium*

RESULTS AND DISCUSSIONS

Isolation of *fusarium* directly from wilt affected soil sample by dilution Plating method

Direct plating of soil sample for isolation of desired pathogen *fusarium* was not so encouraging due to the fact that most soil bacteria grew within 12 to 24 hours to contaminate the plate. Further the saprophytic fungi grew faster and their growth suppresses the appearance and growth of *fusarium* fungus. The low number of *fusarium* colonies in such isolation plates could be due to high contamination from non-*fusarium* fungi or bacteria present in the soil samples as reported by Latiffah Z. et.al. (2010). They further reported that among the three methods they used for isolation of *fusarium* from soil, the best one was the isolation from infected debris and not directly from the soil. Therefore, most of the times the soil borne pathogen particularly the wilt pathogen *fusarium* is isolated from infected root system rather than directly from soil samples. Our results were no different than reported by them, that in soil isolation the plates recover the bacterial colonies first followed by saprophytic fungal growth.

Assessment of use of sliced fruit/vegetable discs and tapioca granules for harvesting of soil borne *fusarium* directly from the soil samples

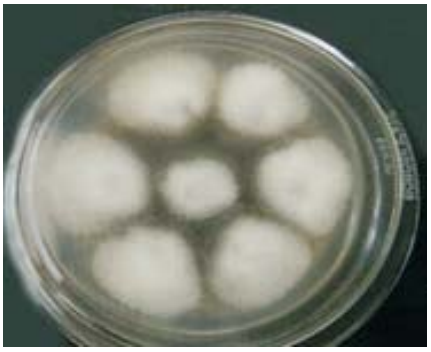
Out of four fruit slice discs and tapioca granules, the tomato slice disc attracted the *fusarium* spore and thus grew into *fusarium* fungal colonies on these, which was confirmed by microscopy (Burgess,1981) (table 1, fig 2.). On other three fruit discs and tapioca granules no *fusarial* growth was observed as confirmed by the microscopy of the fungus. However, these fruit discs favoured the growth of other fungal contaminants.

It was interesting to note that no other fungal growth was favoured and harvested by tomato disc when the soil sample contained the *fusarium* fungus. The *fusarial* spores in the soil suspension attracted towards the tomato disc tissues and formed the web of *fusarial* colony (Figure 2). However, in the absence of *fusarium* fungus in the soil samples, the tomato disc attracts other saprophytic fungi as evident during several isolations for confirmation of the technique. Thus it was concluded that tomato disc was proper among the other fruit discs to harvest soil borne *Fusarium* pathogen from the soil sample.

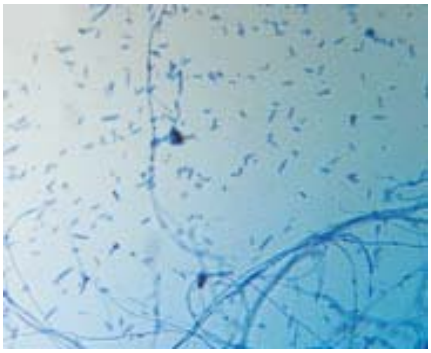
Table 1: Fungal colonies appeared on fruit slice discs and tapioca granules

Fruit slice disc	Fungal growth
Tomato	Adequate growth of *Fusarium*
Cucumber	Growth of saprophytic fungal species
Apple	Growth of saprophytic fungal species
Pear	Growth of saprophytic fungal species
Tapioca granules	Growth of saprophytic fungal species

A: *Fusarium* growth on tomato disc



B: *Fusarium* purified on TAPS media



C: *Fusarium* spores in microscopy

Figure 2: Harvesting of *Fusarium* on tomato disc directly from soil samples and its purification

Furthermore, in the absence of availability of *fusarium* infected root samples or root debris in the soil, the only option in the laboratories is to use the soil sample directly for the isolation of *fusarium* on the special media, but these media also favour the growth of other fungi (Komada, 1975). The selective media for the isolation of *fusarium* is very costly (Mohd Ali et.al, 2014; Andrews and Pitt, 1986). Therefore, it was a need to develop a technique to harvest the wilt pathogen *Fusarium* directly from soil with less contaminants. Harvesting the *fusarium* pathogen on tomato fruit disc is a new and promising technique for isolation of *fusarium* plant pathogen directly from soil and the fungal growth from tomato disc can be further purified on PDA or TAPS media to obtain pure culture of *Fusarium*.

CONCLUSIONS

The isolation of *fusarium* directly from the soil sample, on tomato disc is a new technique of harvesting of *fusarium* from *fusarium* infected soil samples and can be used by plant disease diagnostic labs for assessment of presence of *Fusarial* wilt pathogen in the soil. This is a new and cheap technique of assessment of *fusarium* in the soil samples rather than using a costly specialised media for the isolation of *fusarium,* directly from soil samples.

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