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Management of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing root and stem rot of cucumber by *In vitro* evaluation of bio-efficacy of botanicals: A review

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Abstract
Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases in plants is considered as a substitute method to synthetic fungicides, due to their less negative effects on the human and environment health hazard or implications. The study was conducted to evaluate the antifungal efficacy of few botanicals extracted using water and ether against *Fusarium oxysporum* f. sp. *radicis-cucumerinum in vitro.*

Keywords: Botanicals, water and ether extract, *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

Introduction
Cucumber (*Cucumis sativus* L.) belongs to family *Cucurbitaceae* and most important vegetable, which is major source of human edible products and useful fibers. Cucumber popularly known in India as '*khira*' and gherkins are extensively grown in tropics, subtropics and milder temperate zones of India. In India, major cucumber growing states are Karnataka, Andhra Pradesh, Assam, Bihar, Jammu Kashmir, Telangana, Madhya Pradesh, Orissa, Kerala, Jharkhand and almost all states with total production 1.14 million tons in 78 thousand hectare area (Anon., 2017) [1]. The productivity of the crop is more affected in the polyhouse as well as in field by insects, pest and diseases. Among them, diseases are one of the major constraints affecting quality and quantity of the crop. Many diseases have been reported on cucumbers from different part of the world, but only few of them cause economic losses. Although an accurate estimate is difficult to obtain, the annual crop loss is probably between 20 and 30% (Anon. 2017) [1]. Root and stem rot of cucumber is believed to be caused by a new formae specialis of *F. oxysporum*, presently designated as *F. oxysporum* f. sp. *radicis-cucumerinum* (FORC) (Vakalounakis, 1996) [34]. Root and stem rot is the most destructive disease of glasshouse cucumber crops in Canada in 1994, in France in 1998, in China in 1999 and in Spain in 2000, causing significant losses in the yield (Punja & Parker, 2000) [18]. When cucumber is infected with the root and stem rot fungus, the primary, secondary and tertiary roots and the basal portion of the stem have brown discolorations. On the stem, this discolouration may extend for 40 to 100 cm above the soil line. Fusarium root and stem rot of cucumber has been reported to be favoured at lower soi1 temperatures (17 °C) (Vakalounakis, 1996) [34]. Botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Presence of antimicrobial substances in plant has attracted attention of many research workers in recent years. A number of plants have been shown to have antimicrobial substances. (Spencer *et al.* 1957, Mercer *et al.* 1970, Nicalis 1970, Nene and Kumar 1966, Defoses 1966) [29, 12, 15, 13, 4]. The antifungal properties of plants have been proved in a number of instances as potential means for the control of soil borne disease.

Chemical fungicides are used to control Fusarium wilt of cucumber. Unfortunately, these chemical fungicides are not readily biodegradable; tend to persist for years in the environment and few fungi have developed resistance to them. Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases in plants is considered as a substitute method to synthetic fungicides, due to their less negative effects on

the human and environment health hazard or implications. In the view of above context *In vitro* experiments were conducted to test the antifungal efficacy of some plant at different concentrations against *Fusarium oxysporum* f. sp. *radicis-cucumerinum.*

Material and Method

Evaluation of bio-efficacy of botanicals as water extracts against *F. oxysporum* f. sp. *radicis-cucumerinum* by *In vitro* technique

Fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding a little quantity of sterile distilled water just enough to crush the sample easily. The extract was collected by filtering through the two layers of muslin cloth. Finally, filtrate thus obtained from the leaves was used as stock solution. To study the antifungal mechanism of plant extract, poisoned food technique was followed as suggested by Nene and Thapliyal (1993) [14].

About 20 ml medium was poured into each of the 90 ml sterilized petri plates. Three replications were maintained for each treatment. Suitable control plates were maintained. Each plate was placed with 5 mm mycelial bit aseptically taken from the periphery of 7 days old culture and incubated at 28±2ºC in BOD incubator till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded. The efficacy of plant extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by Vincent (1947) [36].

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

Where

I = Percent inhibition
C = Colony diameter in control;
T = Colony diameter in treatment

Evaluation of bio-efficacy of botanicals as ether extracts against *F. oxysporum* f. sp. *radicis-cucumerinum* by *In vitro* technique

Fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding a little quantity of ether solvent for the extracts of ether-phytoextract. The extract was collected by filtering through the two layers of muslin cloth. Finally, filtrate thus obtained from the leaves was used as stock solution. To study the antifungal mechanism of plant extract, poisoned food technique was followed as suggested by Nene and Thapliyal (1993) [14].

About 20 ml medium was poured into each of the 90 ml sterilized petriplates. Three replications were maintained for each treatment. Suitable control plates were maintained. Each plate was placed with 5 mm mycelial bit aseptically taken from the periphery of 7 days old culture and incubated at 28±2ºC in BOD incubator till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded. The efficacy of plant extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula given by Vincent (1947) [36] and mentioned in water plant extract.

Evaluation of bio-efficacy of botanicals as water and solvent extracts against *Fusarium oxysporum* f. sp. *radicis-cucumerinum* by *In vitro* technique

Khan *et al.* (1974) [9] found the incorporation of oilcakes of *Azadirachta indica* and *Madhuca indica* in the culture medium inhibited the growth of phytopathogenic fungi and the growth reduction was proportional to the concentrations used.

Pradeep *et al.* (1989) [17] tested that the aqueous extract of different part of the plant were tested against plant pathogenic fungi (*Fusarium oxysporum*) were responsible for complete inhibition of spore germination while the rest either stimulated the spore germination or caused partial inhibition of spore germination.

Manasi *et al.* (1990) [11] observed that the extracts from leaves possessed a toxic principle which was effective against *Fusarium maniliform*.

Tariq and Magee (1990) [32] showed that volatile components of crude aqueous extracts of garlic bulb (500 mg/n concentration) inhibited the germination of micro conidia and hyphal extension of *Fusarium oxysporum* f.sp. *lycopersici* in axenic culture.

Shivpuri *et al.* (1997) [24] found extract of *Azadirachta indica* to be highly toxic to *Fusarium oxysporum* f. sp. *ciceris.*

Sindhan *et al.* (1999) [26] plant extract have opened a new avenue for their integration in its management, Besides, being safe and non - phototoxic, use of plant extract is a promising alternative to existing chemical management practices for soil and seed- borne disease.

Singh *et al.* (1999) [28] studied the effect of *Cyperus rotundus* rhizome extract on spore germination, percent germination and germination types of conidia of *F. udum* using different solvent. They recorded increased percent germination with increased in dilution of the extract and was maximum at extract dilution of 1:10 (extract: water).

Bansal and Gupta (2000) [2] found leaf extract of *Azadirachta indica* proved highly toxic to *Fusarium oxysporum*.

Sunil (2002) [30] use the plant products *viz.* Babool seed, Mehandi seeds against *Fusarium oxysporum*· fungi and reported inhibition of mycelial growth and spore germination of these fungi.

Santish *et al.* (2002) [21] tested leaf extract of fourty eight plants for antifungal activity against species of *Fusarium* spp. *Eucalyptus globolus* found effective for mycelial growth inhibition.

Patil (2003) [16] *in vitro* tested various botanicals against *F. oxysporum* causing wilt of patchouli, per cent inhibition achieved was 76.72 per cent with garlic extract (10%) and inhibition of 69.87 per cent tulsi leaf extract (10%) respectively.

Sameer (2003) [20] tested the plant extract of *Boerharia* diffuse *salvadora perisica*, *lptodenia ptirotechica* against the wilt of cumin caused by *Fusarium oxysporum* var. *cumin*i. *Boerhavia* sp. showed maximum inhibition while *salvadora* sp. showed moderate inhibition.

Thakare (2003) [33] evaluated (*in vitro*) the efficacy of some botanicals against *Fusarium oxysporum* and resulted that 100 per cent mycelial growth inhibition was obtained with *Allium sativum* (0.1 per cent) followed by 100 percent with *Azadirachta indica* (10 per cent), 37.48 percent in *Ocimum sanctum* (10 per cent) and 47.97 percent was with *Gliricidia maculata* (10 per cent) respectively.

Verma and Dohroo (2003) [35] showed that garlic cloves extract resulted total inhibition of growth of *Fusarium oxysporum* f. sp. *pisi*.

Riaz *et al.* (2008) [19] *In vitro* tested of some leaf extracts (*Triticum aestivum, Zea mays, Helianthus annus, Capsicum annum, Allium cepa* and *Tagetes erectus*) for antifungal activity at different concentrations (2, 4, 6 and 8% w/v) against *Fusarium oxysporum* f. sp. *gladioli* caused corm rot disease of gladiolus in Pakistan. They observed that extract of *Tagetes erectus, Helianthus annus* and *Capsicum annum* were found highly effective where all the employed extract concentrations significantly reduced fungal biomass by 54-79%, 33-85% and 45-57% respectively.

Sharma *et al.* (2008) [23] developed an ecofriendly antifungal compounds for controlling plant diseases caused by *F. oxysporum*, different extracts of three weed plants, namely, *Capparis decidua, Lantana camara* and *Tridax procumbens*, were tested for their antifungal potential. The spore germination/spore counting technique was followed for the evaluation of the antibiotic properties of the extracts at three different concentrations. Results revealed that the free flavonoids and sterols of *T. procumbens* (flower) and bound flavonoids of *C. decidua* (fruit and stem) totally inhibited spore germination of the fungi (100%).

Singh *et al.* (2010) [27] reported that aqueous extract of *A. indica* was most effective in inhibiting mycelial growth (67.8%) of *F. udum* followed by *Datura festilosa* (61.2%), *Tagetes erecta* (52.6%), *Eucalyptus citridora* (52.2%), *Aegle marmelos* (47.9%) and *Mimusops elengi* (45.9%) respectively.

Chohan *et al.* (2011) [3] registered that toxic effects of five medicinal plants namely, *Azadirachta indica*, *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* and *Allium sativum* were tested at 2, 4, 6 and 8% concentration against *Fusarium oxysporum* f. sp. *gladioli in vitro*. Out of five medicinal plants, extract of *A. indica* showed maximum mycelial growth inhibition both at 8% concentration (83.5) and 2% concentration (34.5%) followed by *T. erecta*, *A. sativum* and *D. stramonium* that suppressed the mycelial growth at 8% concentration *viz.* 58.5, 35 and 28.5% respectively. *O. basilicum* was the least effective in suppressing the mycelial growth of *F. oxysporum.*

Devi and Chhetry, (2012) [5] screened antifungal effect of plant extracts against mycelial growth and spore germination of *F. udum* at different concentrations of 5, 10, 15 and 20 per cent using poisoned food technique and cavity slide method. Among them, *A. sativum* at 20% alone recorded 100% inhibition of mycelial growth and spore germination.

Shukla and Dwivedi, (2012) [25] studied *in vitro* efficacy of different concentration *i.e.* 5, 10 and 15 per cent of plant extracts *viz.*, Bitter guard, Turmeric, Garlic and Black pepper to control *Fusarium udum*. All the plant extracts showed considerable diminution in the growth of pathogens. Growth of *F. udum* has been reduced by 15% concentration of turmeric (89.2%) followed by garlic (88.26%) and black pepper (82.22%).

Abu-Tahon *et al.* (2014) [8] *in vitro* studied the efficacy of 5 medicinal plant extracts *i.e. E. globules, L. camera, Nerium oleander* and *O. basilicum* against *F. oxysporum* f. sp. *lycopersici* race 3 in Egypt and found that cold distilled water extract of *O. basilicum and E. globulus* were most effective to inhibiting the growth of the pathogen.

Anil *et al.* (2015) studied that *F. oxysporum* f. sp. *lycopersici* is an important disease that causes wilt disease in tomato crop world over. Management through chemical fungicides cause serious environmental problems and are toxic to non-target organisms as well. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. In an approach towards the development of eco-friendly management, *In vitro* antifungal assay was conducted against *F. oxysporum* f. sp. *lycopersici* (FOL) using plant extracts of fifteen plants. Out of fifteen plants, three plants proved to be potential in inhibiting the growth of the FOL *viz*., *Solanum indicum* (78.33%), *A. indica* (75.00%), *Oxalis latifolia* (70.33%) at 20% cocentration.

Suryawanshi *et al.* (2016) [31] assessed bio-efficacy of 12 aqueous phyto extracts each at 10 and 15 per cent concentrations against *C. dematium*, applying poisoned food techniques. All the aqueous phyto extracts exhibited antifungal activity against the test pathogen and it was directly proportional to their concentrations. However, significantly highest mycelial growth inhibition was recorded with *A. indica* (60.74%), followed by *A. satium* (56.48%), *O. sanctum* (49.44%) and *A. cepa* (44.85%) and rest of the test phytoextracts recorded mycelial growth inhibition in the range of 18.15 to 45.00%.

Sesan *et al.* (2017) [22] *In vitro* tested nine plant extracts against *F. oxysporum* (strain Fo18) isolated from blackcurrant plants (*Ribes nigrum* L.). The highest growth inhibition 78.6% was recorded for *A. sativum* extract, followed by *Satureja hortensis* and *Valeriana officinalis* extracts (71.4%) at 20% concentration. A good inhibitory activity on mycelial growth has been observed for *Mentha* sp., *Rosmarinus officinalis*, *Hyssopus officinalis* and *Artemisia dracunculus* 'Sativa' (62.8, 58.6, 57.1 and 50% respectively). *Achillea millefolium* extract had no effect on radial growth of *F. oxysporum* isolate. This report is the first in Romania regarding the *In vitro* antifungal activity of some plant extracts on *F. oxysporum* in blackcurrant. These data are very useful for plant protection practice, particularly for medicinal plants, as blackcurrant, which demands for non pollutant and environmental friendly alternative methods to fungicides. Locally plant extracts could have important roles in sustainable based management strategies of *Fusarium* disease in blackcurrant.

Conclusion

To develop effective management strategies, the botanicals should be included in the cultivation practice. Plant extracts are effective in inhibiting the myceial growth of the pathogen in both water and ether extracts. Due to the antimicrobial properties present in the botanical extracts they are very effective in controlling the disease. Hence, the botanicals which were found effective in inhibiting the growth of pathogen and can be incorporated in the management of the disease and can be recommended for use at field level which may help the farmers reducing the cost in use of fungicides and increase in yield which may be attributed by the botanicals applied.

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