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Etiology of Twister Disease Complex in Onion

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A B S T R A C T

In recent years, twister disease of onion has become epidemic in coastal tract and other onion growing districts of Karnataka which caused heavy loss. Survey carried out during *kharif* and *rabi*/summer 2011-12 and 2012-13 revealed typical symptoms of the disease twisting of leaf, neck with blight as well as dieback (anthracnose), scanty root system with galls and showing fungal growth was noticed. Artificial inoculations of onion seedlings with *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Meloidogyne* spp. alone and in combinations expressed twister disease symptoms. Metabolomic changes like increased total sugars and growth hormones (IAA and GA) were seen. Test for pathogenicity demonstrated that twister disease complex whereby the based on all these studies we have proposed a model disease cycle for this twister disease complex.

Introduction

Onion (*Allium cepa* L.) rightly called as "queen of kitchen" is one of the oldest known and an important vegetable crop grown in India. It belongs to the family Alliaceae. Several factors have been identified for the low productivity of onion in India. The most important factors responsible are the diseases like purple blotch, downy mildew, *Stemphylium* blight and now twister disease. Onion twister, a disease of rainy season onion, was first reported near Zaria, north Nigeria, in 1969 (Ebenebe, 1980). Kuruppu, (1999) reported the disease on shallot onions, *Allium cepa* var. *ascalonicum*, that caused yield losses of up to 20 to 30% in Kalpitiya Peninsula in the North Western Province of Sri Lanka. In the 2005-06, this disease has seriously attacked red onion in a number of onion production centers of Indonesia (Wiyono, 2007).

In the recent years, twister disease has become epidemic on onion crop in coastal tract and other onion growing districts in Karnataka. This disease vernacularly in Srilanka called as Disco, in Indonesia Seven whorl and in Karnataka as Haavu suruli roga/Tirupu roga. This disease causing heavy yield loss, leads to shortage in supply to the market resulting in higher prices to a common man. Very less information is available on survey of twister disease of onion in Karnataka. Karwar, Ankola, Kumta, Honnavara and Bhatkal area farmers grow onion in paddy fallow area as

rabi/summer crop with local Kumta variety which is used as table purpose because of its sweet nature. This onion cultivating tract is facing severe twister disease since 2-3 years showing severity up to 40-60 per cent. In Chitradurga, Chikamagaluru and other onion cultivated area also in last two years this disease caused heavy loss to farmers (Hegde *et al.,* 2012 and Nargund *et al.,* 2013).

As there was no clear cut information on etiology, an attempt was made to etiological agent/s involved in the disease development. Hence, the investigation has been taken up to unreal the twists involved in the twister disease of onion

Materials and Methods

Isolation of the pathogen/s

The causal organisms were isolated from onion plants showing the typical twister symptom. The infected parts like leaf sheath, neck and roots were subjected to standard tissue isolation.

Colonies were observed for their morphological and cultural characters. Mass multiplication of isolated fungi in Sand- corn meal medium was prepared in the proportion of 95:5 in order to get maximum inoculum of the fungus. Sand-maize meal. The giant cultures so obtained were used for preparing micro sick plot at the Department of Plant Pathology.

Extraction of root knot nematodes, maintenance and build-up of nematodes inoculum

Cobb's sieving and decanting technique was followed for extraction of nematodes from soil and roots too. The nematode suspension collected in the Petri dish was examined using research stereo binocular microscope. The root knot nematode and other plant parasitic nematodes present in the suspension were identified by observing different morpho-anatomical characters. The galled root system was immersed in a beaker containing boiling 0.1 per cent cotton blue in lactophenol and left overnight for clearing (Hooper, 1986). The roots infected by root knot nematode were washed.

The females were dissected out from the well-developed galls of the roots under the stereo binocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide.

Perineal pattern morphology

Four to ten females from each single-female population were analyzed by perineal pattern Morphology. Perineal patterns were prepared as described in the literature (Starr J.L 2002, Wilson W.R., 1982) and examined under a compound microscope at ×500 and ×640 and photographed.

Tests for pathogenicity

Onion (*Allium cepa*) cv Arka kalyana was used in all experiments. Seeds were thoroughly surface sterilized with one per cent sodium hypochlorite for two minute and washed in sterile water, air dried and sown. The seedlings of 25-30 days were used for further study.

In vivo under pot culture

Thirty days old seedlings of onion cv. Arka kalyana previously grown in a seedbed, were transplanted into earthen pots of size of 20 x 20 cm (diameter x height). At 10 days after transplanting, 4x109spore/ml of ten days old grown on Potato Dextrose Broth (PDB) was sprayed. Sterile distilled water was used for the untreated control.

Interaction studies

Simultaneous inoculation and inoculation after 20 days incubation

C. gloeosporioides (@ 50 ml/pot) +
F. oxysporum (@ 50 ml/pot)

F. oxysporum (@ 50 ml/pot) +
M. graminicola (@ 500 jevannile/pot)

F. oxysporum (@ 50 ml/pot) +
M. graminicola (@ 500 jevannile/pot). +
C. gloeosporioides (@ 50 ml/pot)

To maintain about 100% relative humidity, all inoculated seedlings were covered with polythene bags inside a glasshouse for 48 hours prior to exposure to natural conditions outside. The relative humidity was maintained by spraying the plants with water regularly every day until symptoms were fully developed. Observations were made from onset of disease symptoms to fully development of symptoms. After the plants showed symptoms such plants were carefully uprooted and the fungi were re isolated by standard tissue isolation method. The fungi re isolated were compared with original culture.

Field under sick plot method

Artificial sick plot was developed at the Department of Plant Pathology, College of Agriculture Dharwad by inoculating the mass multiplied cultures of *F.oxysporum* and *M. graminicola* Whereas, for *C. gloeosporioides* was cultured for 10 days at 27 ± 1^0C on PDB. Several acervuli, accompanied by pinkish conidial masses, developed beneath a mat of mycelium, abundant conidia were also produced on the aerial hyphae. The mycelial mats, together with the spore masses were mixed in 500 ml of sterile distilled water in a sterilized Blendor for 60 seconds. Sick plots were prepared in the following way.

Thirty days old seedlings of onion cv. Arka kalyana previously grown in a healthy seedbed, were transplanted in artificially inoculated raised broad base furrow method of bed size 2.5 X 1.2 X 0.15 m. Later, the plants were allowed to establish for one week to avoid transplantation shock if any. Similarly control plants were sprayed with water for comparison. Observations were made regularly for the appearance and development of symptoms. At the end re-isolation was made from the diseased tissues (roots, neck and leaves) from artificially infected plants. The isolate obtained was compared with the original culture for confirmation of fungus under study.

Biochemical changes at different stages of twister disease of onion

Different biochemical compounds were estimated at 0, 3 and 5 grades of disease to know the biochemical compounds induced by pathogens by spectrophotometer (colorimetric assay) and methods used were mentioned below. Protein content was assessed following the Automated Calorimetry method Lowry's method, Phenols by Folin-Ciocalteau reagent (FCR) whereas Total sugars were estimated using the method described by Nelson's modification of Somogyi's method and IAA and GA by following the Automated Calorimetry method.

Results and Discussion

Different symptoms and signs of the disease were noticed on leaves, neck, flowering stalk, inflorescence and also on bulbs were described as below (Plate 1).

Leaf

Twisting, curling and chlorosis symptoms were observed. Circular to oval water-soaked areas and a zone of discoloured tissue was

formed around the spots. Clusters of acervuli were formed in concentric rings in the shallow sunken necrotic spots which were black resembling anthracnose symptom. Further, dieback symptom was noticed in severely affected plants.

Neck and bulb

Elongated neck with slender bulb, which were twisted abnormally.

Root system

Root knot, root discoloration and underdeveloped root system are important symptoms. In few cases, root galling was observed at tip and also intercalary which were whitish in early stage.

Collection and isolation of pathogens from infected plants

The disease samples showing typical fungal symptoms on leaf, neck and root were used for the isolation of the pathogen/s. The pathogen/s were isolated by following standard tissue isolation. In the present study, through tissue isolation technique. *C. gloeosporioides, C. acutatum* and *F. oxysporum* were isolated and the most prevalent and important pathogens throughout Karnataka. The present findings are in accordance with Kuruppu (1999) who reported the association of *Collectotrichum* spp. and *Fusarium* sp in onion twister disease in Sri Lanka

Identification of fungus

The identities of the fungi were done by studying its morphological and growth characteristics and were compared with earlier reports. Based on cultural and morphology of spore, fruiting body and growth parameters was identified by many workers (Jayalakshmi, 2010). The culture isolated from rotted bulbs of onion was identified as *F. oxysporum* based on the morphological and cultural characters as per, Booth (1971).

Identification of nematode

Diagnostic microscopic examination of the galls on roots revealed the presence of eggs, juveniles and female nematode of *Meloidogyne* spp. in the vascular bundles of roots. On an average, 3-4 adult females were present along with immature stages in a single gall. The females were pyriform in shape while the males were filiform. The presence of egg masses outside the gall was a common phenomenon noticed in roots. The egg mass matrix was observed to be whitish, glistening and round which was exposed on the roots

Perineal pattern analysis

Most perineal patterns were typical of *M.graminicola,* as described in the literature.

Pathogenicity tests

Artificial inoculations of onion seedling were carried out as explained in "Material and Methods". Symptoms developed after inoculation were recorded and given in Table 1 and Plate 2 and 3. It is found to be first investigation and results of which are discussed here under. Different inoculation methods were followed for proving pathogenicity. These pathogens were reisolated from infected roots and leaf/sheath and the identities of the causal organisms were confirmed by comparing with the original cultures by standard procedures.

C. gloeosporioides alone

Plants developed symptoms of onion twister disease within 6 DAI, which showed sunken oval lesions on at the necks, these lesions contained clusters of acervuli of

Colletotrichum sp. extended and rotting begins, die back of shoot tip was also observed. These symptoms produced by the pathogen were found to be in agreement with Ebenebe (1980). Inoculation tests of *Colletotrichum* state of *G. cingulata* satisfied Koch's postulates and thus demonstrated causal agent of onion twister disease (Kanlong *et al.*, 1988).

After 96 hours after inoculation, typical onion anthracnose symptoms with salmon coloured mucilaginous spore matrix were observed on the infected leaf surface (Panday *et al.*, 2012).

F. oxysporum alone

Typical onion twister symptoms were seen on stem portion near soil level of 12 days after inoculation (DAI). Elongation of neck from soil level, gradual twisting, progressive yellowing, dieback of the leaf tip, infected bulbs developed white to pinkish mould and premature death.

The infected plants pulled off from soil showed discolouration of roots and complete destruction of root system. The affected plant was died finally due to severe rot. Similar symptoms were reported by Kuruppu (1999) who reported typical symptoms, as observed in the field. Pathogenicity was proved by inoculating the giant culture of *F. oxysporum* to sterile soil and control was maintained without inoculum.

M. graminicola alone

In general, root knot disease caused by *M. graminicola* is one of the major constraints in the productivity of several crops. Out of the several nematodes of economic importance, root knot nematodes are most widely studied and are commonly found involved in synergistic interactions with other fungi. In onion, association of fungus *F. oxysporum* with *M. graminicola* or combination of two or more fungi was also noticed. Hence, studies on pathogenicity aspects were carried out and results are discussed.

Inoculated plants showed stunted growth, yellowing of leaves with slight abnormal elongation with twisting like symptoms in 15 days after inoculation when infected plants were uprooted, root proliferation, slight root galling on bigger roots, were observed. Diagnostic microscopic examination of the galls revealed the presence of eggs, juveniles and females of nematodes in the vascular bundles of roots.

The female was pyriform in shape while the males were filiform and in general outline, differed from the juveniles. The presence of egg masses outside the gall was a common phenomenon noticed in roots. The egg mass matrix was observed to be whitish, glistening and round which were exposed on the roots.

Initially, the possible cause of disease symptoms was suspected as nematodes. However, Kuruppu (1999) isolated the fungus *F. oxysporum* from the diseased shallot plants and reported that thrips, mites, nematodes and other fungi could also cause similar symptoms.

Similarly observations were made by Abawi, *et al.*, (1999) as above-ground symptoms on onions heavily infected with *M. hapla* are those of general stunting, uneven growth, smaller necks and bulbs. The diagnostic symptoms are found on roots as galls or root thickenings of various sizes and shapes.

With respect to rice root-knot nematode, *Meloidogyne graminicola*, infection in rice–onion cropping systems in the Philippines was reported. Gergon (2002) study showed that infected plants had short galled roots, smaller bulbs than normal.

Table.1 Interaction effect of different pathogens associated with twister disease of onion

Treatment	Twister (PDI)	First appearance of symptoms (DAI)	Symptoms observed	Pathogen/ recovered
C. gloeosporioides alone	42.90 (40.88) *	6	Twisting, anthracnose, blight,	C
F. oxysporum alone	36.07 (36.82)	12	Twisting, basal rot	F
M. graminicola alone	7.76 (15.65)	15	Stunting, Twisting, root knot	M
C. gloeosporioides + *F. oxysporum* Simultaneousy	53.92 (47.27)	6	Anthracnose, blight, basal rot	C
M. graminicola + *F. oxysporum* Simultaneousy	30.50 (33.43)	10	Stunting, Twisting, root knot, basal rot	F
Meloidogyne spp + *C. gloeosporioides* Simultaneousy	45.07 (42.15)	12	Stunting, Twisting, blight	C
F. oxysporum + *M. graminicola* + *C. gloeosporioides* Simultaneously	63.17 (52.73)	8	Stunting, Twisting, basal rot, anthracnose	C, F, M
C. gloeosporioides after 20 days inoculation of *F. oxysporum*	81.00 (64.39)	12	Severe Twisting, anthracnose, blight, basal rot	C
M. graminicola after 20 days inoculation of *F. oxysporum*	45.07 (42.13)	15	Twisting, prominent root knot, basal rot	F, M
Meloidogyne spp after 20 days inoculation of *C. gloeosporioides*	64.07 (53.33)	20	Prominent root knot, Twisting, anthracnose	C
Meloidogyne spp after 20 days inoculation of. *F. oxysporum* after 20 days inoculation of *C. gloeosporioides*	88.09 (70.47)	13	Severe Twisting, anthracnose, blight, prominent root knot, basal rot	C, F
IAA	15.84 (23.18)	6	Abnormal Twisting	-
GA	6.60 (14.00)	4	Abnormal elongation	-
IAA+GA Simultaneously	16.09 (23.13)	4	Abnormal elongation and Twisting mimic	-
IAA after 20 days inoculation of GA	34.40 (35.86)	5	Twisting mimic with abnormal growth	-
Untreated control	-	-	No any symptoms	-
S.Em.±	2.54			
CD @ 5%	7.34			

* Arc sine values

Where, C- *C. gloeosporioides*, F- *F. oxysporum*, M – *M.graminicola*

Table.2 Effect of twister disease on metabolomic constituents of onion

Stage of disease	Sugars (µg/g of leaf tissue)			Quantity (µg/g of leaf tissue)			
	Reducing sugars	Non reducing sugars	Total sugars	Total protein	Total phenols	IAA	GA
Healthy (0 grade)	9.32	5.79	15.11	9.92	5.44	5.37	0.63
1st grade	8.10	5.31	13.41	6.34	6.41	4.67	1.67
3rd grade	7.12	5.52	12.34	3.31	6.16	16.50	3.29
5th grade	8.74	6.68	15.54	2.25	5.15	8.57	1.72

Plate.1 Different symptoms and signs of twister disease of onion

Seedling twisting at nursery bed

Colletotrichum blight

Colletotrichum spp. (Acervuli)

Neck twisting

Fusarium sp. (Mycelial mat)

Sclerotium rolfsii (Scleorital bodies)

Whole plant twisting

Seven whorl symptom

Twisting at seed crop

Floral malformation

Plate.2 Proving pathogenisity of *C. gloeosporioides*, *F. oxysporum* GA, IAA and *M.graminicola* alone symptoms produced in different stages

Abnormal elongation at basal portion

Neck twisting

Abnormal elongation at tip of leaf

Top portion lodging

Abnormal elongation at tip (GA)

Twisting of leaves

Proliferation of leaves

Acervuli at neck portion

Dieback symptom

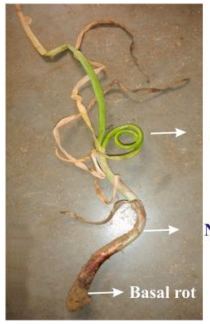
Abnormal proliferation of leaves

Abnormal elongation at basal portion (IAA)

Rotting of bulb

Spore balls in culture plate

Acervuli on rotten bulb



Typical twisting symptom

Root knot (*Meloidogyne* spp.)

Plate.3 Proving pathogenisity produced *C. gloeosporioides*, *F. oxysporum* and *M.graminicola* in combination

C. gloeosporioides

F. oxysporum

Meloidogyne spp. + *F. oxysporum*

F. oxysporum + *C. gloeosporioides*

Meloidogyne spp.

F. oxysporum + *C. gloeosporioides*

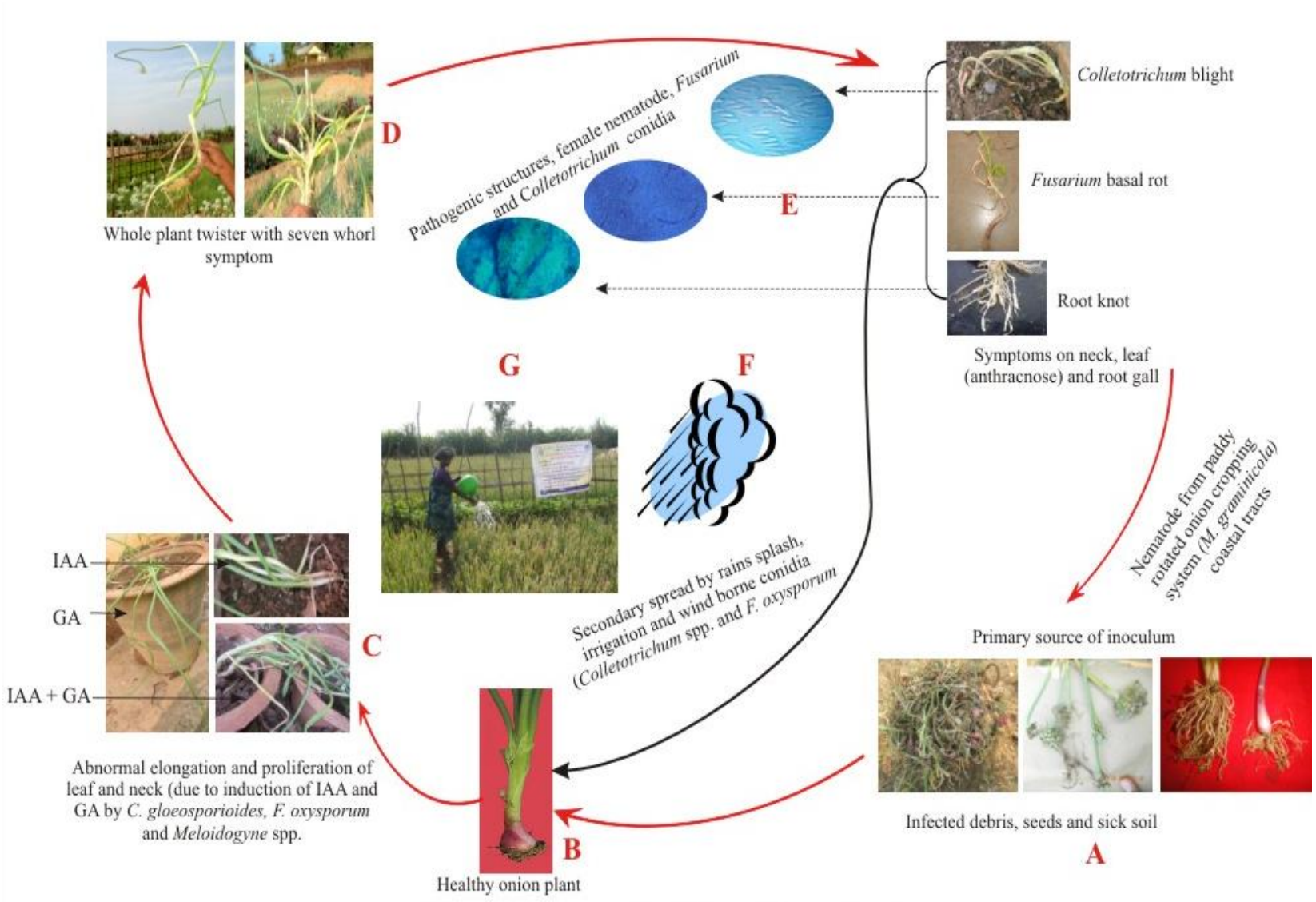
Meloidogyne spp. + *F. oxysporum*

Meloidogyne spp. + *C. gloeosporioides*

Meloidogyne spp. + *F. oxysporum* + *C. gloeosporioides*

Meloidogyne spp. + *F. oxysporum* + *C. gloeosporioides*

Plate.4 Disease cycle of twister disease of onion



Details of treatments were mentioned below

Treatment		Concentration
T_1	*C. gloeosporioides* alone (4x109spore/ml)	50 ml/pot
T_2	*F. oxysporum* alone (4x109spore/ml)	50 ml/pot
T_3	*M. graminicola* alone (Jevannilel suspension)	500 jevannile/pot
T_4	IAA (Indole acetic acid)	200 ppm
T_5	GA (Gibberlic acid)	200 ppm.
T_6	IAA + GA	200 ppm.
T_7	Untreated control	

Field under Sick plot method

T_1	**Inoculation of *C. gloeosporioides* (@ 500ml/plot) after 30 days *F. oxysporum* (@ 500 ml/plot)**
T_2	Inoculation of *F. oxysporum* (@ 500ml/plot) after 30 days later *M. graminicola* (@ 500 jevannile/plot)
T_3	Inoculation of *F. oxysporum* (@ 500 ml/plot) after 30 days later *M. graminicola* (@ 500jevannile/plot). After 30 days *C. gloeosporioides*. (@ 500 ml/plot)

IAA

Plants showed abnormal elongation of leaf on 6th day after spraying of IAA @ 200 ppm. Further, neck region also showed elongation with twisting of 15.84 per cent. These plants didn't show any pathogen structures.

GA

First symptom appeared on 6th day after spray of GA @ 200 ppm which showed at the tip of leaf curling, increased leaf and stem length with 6.60 per cent twisting. These sprayed seedlings didn't show any pathogen structures too.

Interaction studies

Studies on host-pathogen interaction aimed at studying various symptoms produced by each of the pathogen in combination and it revealed that symptom, expressed very early but, rapid twisting was observed and showed aggravation of the disease.

C. gloeosporioides after 20 days inoculation of *F. oxysporum*

In this combination severe twisting and anthracnose at leaf and neck were observed with basal rot. Due to rotting of stem, die back plants showed discolouration of roots and complete destruction of root system. The affected plant was killed finally due to severe rot with PDI of 81.00. These findings are in conformity with those recorded by Mani and Sethi (1987) who worked on chick pea wilt. Similar reports were on *C. gloeosporioides* and *Gibberilla moniliformis* in onions for twister disease. Both *C. gloeosporioides* and *F. oxysporum* f. sp. *cepae* from the diseased plants were collected at Kalpitiya.

Inoculation of *F. oxysporum* after 20 days inoculation of *M. graminicola*and after 20 days inoculation of *C. gloeosporioides*

The first symptoms appeared at 20 DAI characterized by abnormal elongation, curling chlorosis and abnormal elongation of neck

and slender bulbs. These lesions enlarged all over the leaf. Black, minute, slightly raised acervuli with pink masses of conidia could be seen scattered on the surface of the lesions. Yellowing and dieback of the leaf tips withered. Bulbs produced from these plants were small root deformed with galls of varying size were noticed. The infected bulbs showed rotting before harvest and developed a symptomatic white to pinkish mould and with PDI of 88.09.It is a new report on involvement of *M. graminicola* with *F. oxysporum* and *C. gloeosporioides* as an etiological agent for twister disease complex in Karnataka.

IAA after 20 days inoculation of GA

Plants showed symptom on 5th day with abnormal elongation at the tip leading to irregular leaf curling, twisting and proliferation of leaf and stem with final twisting of 16.09. Here also plants exactly mimic twister symptoms but didn't show any pathogen structures too. Ongoagwanit (1991) used the same procedures for detection of auxins when applied to the onion plants.

He reported that role of IAA in twister disease and its concentration was higher in diseased plants compared to corresponding healthy plants. Application of IAA, GA and their combination on onion seedlings revealed the twisting symptoms appearance. Further severity of twisting was enhanced when used in combinations (GA + IAA) compared to their individual spray. Synergistic effect in symptom expression was observed in combination. This clearly indicate the role of *C. gloeosporioides*, *F. oxysoprium* and *M. graminicola* have brought out change in concentration of IAA or GA or both directly as these pathogens have induced or produced these hormones in disease development. This is confirmed by absence of any twister symptoms in untreated healthy seedlings.

Metabolomic changes at different stages of twister disease of onion

Metabolomic changes at different stages of disease development mechanisms were analysed for reducing, non-reducing and total sugars, total proteins, total phenols and growth regulators like IAA and GA.

The reducing sugar, non-reducing sugar and total sugar were higher in 5th grade (8.74, 6.68 and 15.54 □ □ g/g of leaf tissue) whereas, total proteins were higher in 1st grade (9.92 □ □ g/g of leaf tissue). As for as growth regulators are concerned IAA and GA were highest in 3rd grade (16.50 and 3.29 □ □ g/g of leaf tissue). The HPLC analysis of IAA content was higher in 3rd grade diseased leaves which is of 5.14□ g/g of leaf tissue whereas, lowest in healthy seedling stage (2.38□ g/g of leaf tissue). The GA content was higher in 3rd grade diseased leaves were which is of 4.17□ □ g/g of leaf tissue. Whereas lowest in healthy (3.16□ g/g of leaf tissue)Table 2.

Auxin is a pivotal plant hormone that regulates many aspects of plant growth and development. Auxin signaling is also known to promote plant disease caused by plant pathogens. However, the mechanism by which this hormone confers susceptibility to pathogens is not well understood. Involvement of fungal plant pathogens in host auxin metabolism in *Arabidopsis thaliana* showed that IAA-Asp increases pathogen progression in the plant by regulating the transcription of virulence genes. These data highlight a novel mechanism to promote plant susceptibility to pathogens through auxin conjugation (González-Lamothe *et al.*, 2012). The metabolic products of *C. gloeosporioides* infection i.e., IAA was more in concentration in diseased plants than that of the healthy plants (Ongoagwanit, 1991). The standard IAA, 200 ppm tryptophan caused an abnormal symtoms of elongation and twisting of young

leaf in seven days (Wongviset, 1995 and Alberto R.T., 2014).

Disease cycle of twister disease of onion

Different pathogens were encountered during the cause of investigation. The survival/ overwintering, and their infection has significance in disease cycle. Further, spread and possible weather factors that influence the disease cycle has been envisaged in disease cycle (Plate 4).

It indicated that the aforementioned pathogens survive in the infected debris, bulbs and seeds as dormant mycelium or spore. As for as the perfect states *viz., Glomerella cingulata, G.acutata* for *Colletotrichum* spp, *Gibberella moniliformis* for *F. oxysporum* respectively has been established. The primary infection was noticed in the nursery bed in transplanting onion and in seedling stage in direct sown crop. Primary source of inoculum is seed borne, soil borne and plant debris for *C. gloeosporioides* and *F. oxysporum.* In case of *M.graminicola* it is mainly soil borne and in the cropping pattern of paddy-onion which is very common in coastal Karnataka it perpetuates on both the crops. Further *M.graminicola* has some weeds as host range (gramenatious grasses). Infection by *F. oxysporum* induces the production of gibberlic acid (GA) leads to abnormal elongation at the leaf tips. In case of *C. gloeosporioides* which induce indole acetic acid (IAA) production leading to abnormal elongation at the neck region. However, *M.graminicola* induces both the above mentioned growth hormones. Various kinds symptoms viz abnormal elongation of leaf tip, neck and bulb. In the later stages anthracnose symptoms on above ground parts end with dieback. Whereas in below ground parts root system with prominent galling, discoloration and proliferation in severe cases basal rot symptoms with pinkish fungal growth (*F. oxysporum)* was also observed. In seed crops, malformation of inflorescence (umbel) leading to poor seed development and also serves as source of inoculum to next season. The secondary spread of inoculum in fungal pathogens (*C. gloeosporioides*. and *F. oxysporum*,) is brought by irrigation water, sprinkler, wind accompanied by rain, rain splash, manual sprinkling of water (very common in coastal Karnataka).

This is for first kind in showing the complete disease cycle of onion twister disease globally. However, Jamadhar (2007) has reported disease cycle of similar nature in anthracnose of grape.

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