

“ECO-FRIENDLY MANAGEMENT OF CASTOR (*Ricinus communis* L.) WILT CAUSED BY *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad IN SOUTH GUJARAT CONDITION”

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**BIPINCHANDRA VAHUNIA
B.Sc. (Hons.) Agriculture**

**DEPARTMENT OF PLANT PATHOLOGY
N.M. COLLEGE OF AGRICULTURE
NAVSARI AGRICULTURAL UNIVERSITY
NAVSARI- 396 450
GUJARAT STATE**

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Abstract

**Eco-friendly management of castor (*Ricinus communis* L.)
wilt caused by *Fusarium oxysporum* f. sp. *ricini* Nanda and
Prasad in South Gujarat condition**

Name of Student

Major Advisor

BIPINCHANDRA VAHUNIA

Dr. PUSHPENDRA SINGH

**DEPARTMENT OF PLANT PATHOLOGY
N. M. COLLEGE OF AGRICULTURE
NAVSARI AGRICULTURAL UNIVERSITY
NAVSARI**

ABSTRACT

Castor (*Ricinus communis* L.) is a most important non-edible oilseed crop of arid and semi-arid regions of India. Gujarat is the leading castor growing state having maximum area, production and productivity. Wilt of castor caused by *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad is a serious problem in Gujarat state and causes heavy losses. Considering the serious threat to castor cultivation, the present investigation was carried out on various pathological aspects to generate scientific information of pathogen and effective source of disease control. The studies included identification of causal organism, evaluation of biological control agent, botanicals, extract of organic amendment *in vitro* and pot culture conditions.

The tissue isolation from stem of infected plant revealed the association of *Fusarium* sp. which was identified as *Fusarium oxysporum* f. sp. *ricini*. The pathogenicity of the fungus was confirmed by soil inoculation that successfully produced wilt symptoms and found pathogenic.

Seven known antagonist were tested *in vitro* for their antagonism against *Fusarium oxysporum* f. sp. *ricini* by dual culture method. *Trichoderma harzianum* (72.22%), *Trichoderma viride* (70.37%) and *Pseudomonas fluorescens* appeared potent antagonist followed by *T. faciculatum*, *T. longibrachyatum*, *T. koningii* and *Bacillus subtilis*.

The botanicals of commonly available seven plant species were evaluated *in vitro* by poison food technique against *Fusarium oxysporum* f. sp. *ricini*. The extract of turmeric rhizomes (*Curcuma longa* L.) was effective in inhibiting mycelial growth 42.22 Per cent followed by 36.67 Per cent inhibition by leaves extract of marigold (*Tagetes erecta* L.).

The seven organic manure/cakes extract were tested against *Fusarium oxysporum* f. sp. *ricini* by poison food technique *in vitro*. Significantly minimum growth (44mm) mycelium was recorded in neem cake followed by Poultry manure (46.67mm).

The effective biocontrol agents, botanicals and organic manure/cakes which were found effective under laboratory studies were further evaluated under pot condition. Seed treatment with *Trichoderma harzianum*@ 6g/kg followed by

Trichoderma viride@ 6g/kg were found effective for the management of castor wilt.

Dr. PUSHPENDRA SINGH

Associate Professor (Plant Pathology),

ASPEE SHAKILAM Agri Biotechnology Institute,

N.A.U., Surat- 3950070

CERTIFICATE

This is to certify that the thesis entitled “**ECO-FRIENDLY MANAGEMENT OF CASTOR (*Ricinus communis* L.) WILT CAUSED BY *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad IN SOUTH GUJARAT CONDITION**” submitted by **BIPINCHANDRA VAHUNIA** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in the subject of **PLANT PATHOLOGY** of the **NAVSARI AGRICULTURAL UNIVERSITY**, is a record of bonafied research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Navsari

(PUSHPENDRA SINGH)

Date:

Major Advisor

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. PUSHPENDRA SINGH**, Associate Professor (Plant Pathology), ASPEE SHAKILAM Agri Biotechnology Institute, Surat and no part of the work has been submitted for any other degree so far.

Place: Navsari

Date: **(BIPINCHANDRA VAHUNIA)**

Countersigned by

(PUSHPEDRA SINGH)

Associate Professor (Plant Pathology)
ASPEE SHAKILAM Agri Biotechnology Institute,
Navsari Agricultural University
Surat-3950070

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Place: Navsari

Date:

(Bipinchandra Vahunia)

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Introduction

I. INTRODUCTION

Castor (*Ricinus communis* L.) is one of the most important non-edible oilseed crops of arid and semi-arid regions of India. It belongs to genus *Ricinus* of *Euphorbiaceae* family and its common name is castor bean, other common names include castor-oil plant and palma christi. Its sanskrit name “Erandh” indicates the property of the drug to dispel diseases. Castor is indigenous to Eastern Africa and most probably originated in Ethiopia. The castor plant has been cultivated for centuries for the oil produced by its seeds. Among the non-edible annual group of oilseed crops, castor constitutes very important position in the oil seed economy throughout the world. It is grown in tropical and sub-tropical climate.

The major castor growing countries are India, China, Brazil, Russia, Thailand and Philippines. Among these, India is the largest castor producer in the world. The area, production and productivity of castor in India during 2014-15 were 10.63 lakh hectares, 17.27 lakh tones and 1624 kg/ha, respectively (Anon., 2015). The major castor growing states in India are Gujarat, Rajasthan, Andhra Pradesh, Tamilnadu, Karnataka, and Orissa. Gujarat ranks first in area and production, contributing about 80 per cent of the country’s area and production. In Gujarat, 13.95 lakh tones of castor seed were produced from an area of 7.34 lakh hectares with an average productivity of 1900 kg/ha during 2014-15 (Anon., 2015). In Gujarat, castor is mainly grown in nine districts of

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which Banaskantha, Mehsana and Sabarkantha are the major castor growing districts. Castor is a long duration crop, where sowing starts in July-August and harvested in February-March.

Castor seed contain 40-55% of oil which has uses range from cosmetics, paints, synthetic resins and varnishes, to the areas of national security involving engineering plastics and polymers for electronics and telecommunication. Castor is a versatile, renewable resource having vast and varied application such as surface coatings, telecom, pharma, rubber chemicals, nylons, etc. Castor oil and its derivatives find major application in soaps, hydraulic brake fluids and polymers and perfumery products. The primary use of castor oil is as a basic ingredient in the production of nylon 11, coating and inks, surfactants, polyurethanes, polishes, flypapers and many other chemical derivatives and medicinal, pharmaceutical and cosmetic derivatives. Castor oil is obtained by pressing the seeds, followed by solvent extraction of the pressed cake. Castor oil is one of the world's most useful and economically important natural plant oils.

Castor seed oil cakes is a very good fertilizer alternative containing optimum levels of Nitrogen, Phosphorus and Potassium which is suitable for cultivation of paddy, wheat, maize and sugarcane.

Due to high protein content (12–15 %), the cake is used as a binder in the production of plywood, match boxes and packing boxes. The plant stalks are used in manufacturing papers, card boards, hut making and as fuel.

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The refined oil also has a good domestic market. The castor oil is different from other vegetable oils in the sense that it does not freeze even under adverse temperatures of -12 to -18°C. It is therefore, considered as the best lubricating agent particularly for both high speed engines and aeroplanes. As a result, demand for castor oil inside and outside the country has increased with the advancement of industrialization all over the world. The castor plants are grown for rearing “eri” silkworm for the production of “eri” silk. Castor cake is widely used as manure as it contains considerable amount of nitrogen (4.5 to 5.5%) and other mineral elements. However, it is poisonous as animal feed because of the presence of a protein “ricin” (blood coagulating factor) and toxic alkaloid “ricinine” (Damodaran and Hegde, 2002).

Most of the castor cultivars have been developed by hybridization and subsequent selection. Gujarat is pioneer in development and release of first commercial hybrid GCH 3. Since then, exploitation of hybrid vigour has become routine in castor and subsequently more improved hybrids like GAUCH 1, GCH 2, GCH 4, GCH 5, GCH 6 and GCH 7 have been released during the spell of forty years. The production is highest in Gujarat state because most of the farmers are growing hybrids under irrigated conditions. The hybrids are well accepted by the farmers because of their high production potentiality over local varieties and the crop becomes very remunerative to the farmers.

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Castor is affected by several fungal, bacterial and viral diseases. The important fungal diseases are Phytophthora seedling blight (*Phytophthora nicotianae* BdeHaan var. *parasitica*), Rust (*Melampsora ricini*), Alternaria leaf spot (*Alternaria ricini*), Cercospora leaf spot (*Cercospra ricinella* Sacc. and Beslese), Anthracnose (*Colletotrichum gloeosporiodes*), Powdery Mildew (*Leveillula taurica*), Stem rot (*Macrophomina phaseolina*), and Fusarium wilt (*Fusarium oxysporum* f. sp. *ricini*). Important bacterial diseases are Bacterial leaf spot (*Xanthomonas compestris* pv. *ricini*), Bacterial wilt or Slime disease (*Pseudomonas solanacearum*) and viral diseases are like Tobacco ring spot, Tobacco necrosis and cucumber mosaic virus. Earlier, wilt was considered as a disease of minor important but now it has become a major impediment in the commercial cultivation of crop. Castor wilt was first time recorded in Morocco (Reiuf, 1953). Later wilt disease was reported in erstwhile Russia and causal agent is also identified as *Fusarium oxysporum* f. sp. *ricini*. In India, wilt was recorded for the first time from Udaipur and Sirohi districts of Rajasthan by Nanda and Prasad (1974) and later from Gujarat during 1980-81. The causal organism was established as *F. oxysporum* f. sp. *ricini*. The extent of disease incidence was up to 80 per cent in erstwhile Russia (Moshkin, 1986). Losses in yield were observed in all cultivated castor hybrids in Gujarat. Wilt caused by *F. oxysporum* f. sp. *ricini* is mainly soil borne and also seed borne disease accounting to a loss of 10-20% (Chattopadhyay, 2000). The disease appears at all growth stages of the crop but

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more conspicuous during flowering and spike formation stage. The disease assumed serious proportion in all the castor growing areas due to prolonged survival of the pathogen in the soil and susceptibility of popular cultivars of the crop.

Wilt is a serious problem in castor cultivation in Gujarat. Systematic work has not been done so far on this important pathogen under South Gujarat condition. Looking to the seriousness of this disease and economic important of the crop in this area. Generate necessary information for suitable management measure to minimize crop losses and developing scientific information on the following objectives

- [1] Efficacy of native bio-agents against *Fusarium oxysporum* f. sp. *ricini*
- [2] Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*
- [3] Effect of different organic manure/cakes against *Fusarium oxysporum* f. sp. *ricini*
- [4] Management of wilt under pot condition



Review
of
Literature

II. REVIEW OF LITERATURE

Plant disease is one of the major constraints in the crop production as they inflict heavy losses. Castor is affected by many fungal diseases *viz.*, Phytophthora seedling blight (*Phytophthora nicotianae* BdeHaan var. *parasitica*), Rust (*Melampsora ricini*), Alternaria leaf spot (*Alternaria ricini*), Cercospora leaf spot (*Cercospra ricinella* Sacc. and Beslese), Anthracnose (*Colletotrichum gloeosporiodes*), and Fusarium wilt (*Fusarium oxysporum* f. sp. *ricini*). Important bacterial diseases are Bacterial leaf spot (*Xanthomonas compestris* pv.*ricini*), Bacterial wilt or Slime disease (*Pseudomonas solanacearum*) and viral diseases are like Tobacco ring spot, Tobacco necrosis and cucumber mosaic virus.

Castor crop was found severely affected by *Fusarium* wilt at Navsari and surrounding area during season posing a serious threat.

2.1 Isolation, Identification and Pathogenicity of pathogen:

Gerdemann and Finley (1950) isolated 55 isolated of *Fusarium oxysporum* f. sp. *lycopersici* from diseased tomatoes growing in field on the university farm, Miaaouri (Columbia).

In India, the castor wilt was recorded for the first time in 1974 from Udaipur (Rajasthan) by Nanda and Prasad (1974) and causal organism was established as *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad.

Ram and Pandey (2011) isolated *Fusarium udum* from infected plant of pigeon pea, collected from farmer field of Bharuch and Vadodara district and maintained on potato dextrose agar.

Ramaiah and Garampalli (2015) isolated *F. oxysporum* f. sp. *lycopersici* from infected plant of tomato growing in Mysore region and maintained on potato dextrose agar.

2.2 Efficacy of native bio-agents against *Fusarium oxysporum* f. sp. *ricini*

Antagonism among the microorganisms is now well known and exploited in biological control of plant pathogens.

Pushpavathi *et al.* (1998) studied antagonistic efficacy of different fungal bioagents through dual culture technique. *Trichoderma harzianum*, *T. viride*, *Gliocladium virens*, *Aspergillus flavus* and *A. niger* were screened for their antagonistic activity against castor wilt pathogen (*Fusarium oxysporum* f. sp. *ricini*) and reported that the growth reduction of *Fusarium oxysporum* f. sp. *ricini* was maximum (66.19%) by *T. harzianum*.

Madhukeshwara and Seshadri (2001) reported that maximum inhibition of mycelial growth of *Fusarium udum* was observed with *Trichoderma viride* (83.03%) which was followed by *Pseudomonas fluorescens* (79.07%) and *Bacillus subtilis* (68.14%) in dual culture method.

Landa *et al.* (2001) evaluated *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Stenotrophomonas* spp. Out of 23 bacterial isolates tested, 21 isolates inhibited *F. oxysporum* f. sp. *ciceri*

in vitro. The strains RGAF 19 and RG 26 of *P. fluorescens*, which did not inhibit the pathogen, showed the greatest fusarium wilt suppression.

Jayalakshmi *et al.* (2003) reported that maximum inhibition of- mycelial growth of *Fusarium udum* was observed with local isolate (L1) of *Trichoderma harzianum* (88.69%). *Trichoderma koningii* (D) inhibited the growth of the pathogen to an extent of 77.42 per cent over control. The other bioagents, *viz.*, *Trichoderma sp.* (R), *Trichoderma harzianum* (C) *Trichoderma viride* (D), *Trichoderma harziaunm* (D), *Trichoderma hamatum* (D) and *Trichoderma pseudokoningii* (D), reduced the mycelial growth of the *Fusarium udum* by 76.20, 74.62, 71.79, 66.17, 63.38 and 53.33 per cent, respectively, by dual culture technique and in *in vitro* conditions.

Sharma *et al.* (2003) studied antagonistic activity of eight species of *Trichoderma viz.*, *T. viride* (K), *T. koningii* (P), *T. harzianum* (R), *T. harzianum* (P), *T. hamatum* (P), *T. longorum* (P), *T. viride* (P) against *Fusarium oxysporum* f. sp. *lini* inflicting wilt of linseed under *in vitro* and *in vivo* conditions. In *in vitro* conditions, maximum inhibition of *F. oxysporum* f. sp. *lini* was recorded by *T. viride* (P) (60.53%) followed by *T. harzianum* (P) (47.37%).

Nikam *et al.* (2007) reported that maximum inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* with *Trichoderma harzianum* (83.33%) followed by *Trichoderma viride* (76.66%) and *T. hamatum* (67.77%) in dual culture method.

REVIEW OF LITERATURE

Ramezani (2009) found that all the six bioagents inhibited the growth of *Fusarium oxysporum* f. sp. *ciceri* by dual culture technique in *in vitro* conditions. After 7 days of inoculation *Trichoderma harzianum* exhibited the maximum biocontrol activity causing an inhibition zone of 17 mm followed by *Gliocladium virens*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma viride* and *Trichoderma hamatum* causing 14, 13, 12, 10 and 7 mm inhibition zones, respectively.

Niranjana *et al.* (2009) evaluated *Trichoderma harzianum* and *Pseudomonas fluorescens* isolates against *Fusarium udum* Butler by dual culture technique. They found maximum inhibition zone of 2.2 cm was measured for *Pseudomonas fluorescens* isolate 7 and minimum zone of 0.2 cm was found for isolate 8. For *Trichoderma harzianum*, a maximum of 63 per cent growth inhibition of *Fusarium udum* was recorded for isolate 4 and a minimum of 24 per cent was recorded for isolate 10.

Mehta *et al.* (2010) observed that maximum growth inhibition of *Fusarium udum* was recorded in *Trichoderma viride* (74.04%) followed by *Trichoderma harzianum* (70.22%), *T. longibrachyatum* (61.06%) and *B. subtilis* (50.38%) by dual culture technique.

Singh *et al.* (2010) observed that maximum growth inhibition of *Fusarium udum* was recorded in *Trichoderma viride* (83.48%) followed by *Trichoderma harzianum* (35.80%) by dual culture technique.

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Ram and Pandey (2011) studied *Trichoderma viride* completely inhibited the mycelial growth of *Fusarium udum* in dual culture and was found superior over bacterial bioagent. *Pseudomonas fluorescens* reduced the growth of the pathogen 30.18 mm as compared to 85.68 mm in control.

Zacharia *et al.* (2012) reported that *Trichoderma viride* was highest inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceri* at the ratio 1:4 (60.49%) followed by 1:2 (31.94%).

Athul *et al.* (2012) studied the effect of *Trichoderma* isolate and *Pseudomonas* isolate against wilt (*Fusarium oxysporum*) of vanilla (*Vanilla planifolia*). For this, 10 predominant isolates of *Trichoderma* spp. From vanilla growing area are used and found that isolate T5 showed maximum inhibition ($87.76\% \pm 0.15$) followed by the isolate T2 ($84.17\% \pm 0.38$). On other hand 10 isolate of *Pseudomonas* tested for their bio control potential against fungal pathogen and result revealed that isolate P7 showed maximum inhibition against *Fusarium oxysporum* ($60.24\% \pm 0.226$) followed by P4 ($55.41\% \pm 0.33$).

Prasad *et al.* (2012) reported that maximum inhibition of mycelial growth of *Fusarium udum* was observed with *Trichoderma viride* (91.13%) which was followed by bacterial bioagent *Bacillus subtilis* (88.33%) and *Pseudomonas fluorescens* (71.29%) in dual culture method.

Devi and Chhetry (2012) observed that maximum growth inhibition of *Fusarium udum* was recorded in *Trichoderma*

viride (78.30%) followed by *Trichoderma harzianum* (66.60%) by dual culture technique.

Naz *et al.* (2013) observed that maximum growth inhibition of *Fusarium oxysporum* f. sp. *psidii* was in *Trichoderma harzianum* (57.80%) and bacterial bioagent *Pseudomonas fluorescens* (36.80%) by dual culture technique.

Sundaramoorthy and Balabaskar (2013) evaluated fifteen native isolates of *Trichoderma* spp. for their *in vitro* antagonism against the *Fusarium oxysporum* f. sp. *lycopersici* by dual cultural technique. The results indicated that ANR-1 inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* to an extent of 53.00 per cent over control. This was followed by KGI-3 (38.12 %), RTM-5 6 (31.11%) and KPI-9 (27.22 %).

Hossain *et al.* (2013) tested 20 isolate of *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *ciceri* on PDA by dual culture technique. All the isolates of *Trichoderma harzianum* caused significant reduction in the mycelial growth of the pathogen *in vitro* compared to the control. Isolate T-75 showed the highest (75.89%) reduction of the radial growth of *Fusarium oxysporum* f. sp. *ciceri* followed by T-3 (70.33%), T-12 (70.33%), T-20 (69.22%) and T-25 (68.11%). The lowest radial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* was observed by the isolate T-9 (55.56%).

Rehman *et al.* (2013) indicated that the combined effect of both antagonists (*Trichoderma viride* + *Trichoderma harzianum*) was found to be most effective (87.33%) in

inhibition of *Fusarium oxysporum* f. sp. *ricini* growth as compared to the control followed significantly by *Trichoderma harzianum* (73.33%) and *Trichoderma viride* (60.00%) in *in vitro* condition by dual culture technique.

Meghwal *et al.* (2014) Maximum inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ricini* was observed with *Trichoderma harzianum* (71.27 %) which was followed by *T. viride* (68.15%) and *T. koningi* (63.98%) by dual culture method.

Mailem *et al.* (2015) reported that maximum inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* was observed with *Pseudomonas fluorescens* (83.30%) which was followed by *Trichoderma viride* (79.30%), *T. harzianum* (75.90%) and *B. subtilis* (67.20%) in dual culture method.

Patil *et al.* (2015) reported that maximum inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* was observed with *Trichoderma viride* (80.10%), followed by, *Trichoderma koningii* (64.00%), *Trichoderma harzianum* (62.40%), *G. virens* (61.00%), and *Trichoderma pseudokoningii* (47.00%); whereas, it was significantly least with *Pseudomonas fluorescens* (35.00%) by dual culture technique.

Kala *et al.* (2016) reported that maximum inhibition of *Fusarium oxysporum* f. sp. *ciceri* was observed with *Pseudomonas fluorescens* (84.10%) followed by *Trichoderma harzianum* (72.25%) and *Trichoderma viride* (71.96%) by dual culture technique in *in vitro* conditions.

2.3 Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*

Dwivedi and Shukla (2000) found that phytoextract of *Ocimum sanctum* at 100% concentration inhibited the spore germination of *Fusarium oxysporum* species by 16 per cent.

Irum Mukhtar (2007) studied the antifungal effect of aqueous extracts of four plant species viz., *Azadirachta indica*, *Datura metel* L., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. under *in vitro*. It was found that all the plant extracts at 40% concentration were effective in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. Among these plants extracts, *A. indica* and *D. metel* inhibited fungal growth by 80 per cent. Even at 10 per cent concentration, both plants extracts had inhibitory effect, while *O. sanctum* extracts showed low inhibition (60%) as compared to other plant extracts.

Mehta *et al.* (2010) studied *in vitro* evolution of fourteen plant extract against *Fusarium udum* and found growth inhibition in Turmeric (*Curcuma longa* L.) (58.40%) and Tulsi (*Ocimum sanctum* L.) (10.40%) at 5 per cent concentration.

Singh *et al.* (2010) found that fresh leaf extract of *Tagetes erecta* at 5 per cent concentration effective in inhibiting the 52.6 per cent mycelial growth of *Fusarium udum* in *in vitro* condition.

Singh and Kumar (2011) evaluated eight plant extracts at 1 and 3 per cent concentration against *Fusarium oxysporum* f. sp. *chrysanthemi* among them *Datura stramonium* inhibited

(43.60%) and (56.80%) mycelial growth of *Fusarium oxysporum* f. sp. *chrysanthemi* respectively using poison food technique in *in vitro* conditions.

Shukla and Dwivedi (2012) observed that growth of *Fusarium udum* has been reduced to 78.52% at 5% concentration, 82.22% at 10% concentration and 89.22% at 15% concentration with extract of turmeric (*Curcuma longa* L.). In case of *Fusarium oxysporum* f. sp. *ciceri* growth of pathogen has been reduced by 74.96%, 85.04% and 87.96% at 5%, 10% and 15% concentration with extract of turmeric in *in vitro* condition respectively by poison food technique.

Singh *et al.* (2014) observed that plant extract of Turmeric, Datura, Ginger, Tulsi and Marigold at 10% concentration inhibit the mycelial growth 38.15%, 64.26%, 32.41%, 32.22% and 16.48%, respectively of *Fusarium oxysporum* f. sp. *lini*. in *in vitro* condition.

Shalini *et al.* (2015) observed that plant extract of Tulsi (*Ocimum sanctum* L.), turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinalis* Rosa.) at 5% concentration inhibit the mycelial growth 80.83%, 68.05% and 45.92% respectively, *Fusarium oxysporum* f. sp. *ricini* in *in vitro* condition.

Chohan and Perveen (2015) found that phytoextract of *Zingiber officinalis* and *Curcuma longa* at 20,40,60 and 80 per cent concentration inhibited the mycelial growth ranging from 30.33 to 44.49 per cent and 32.93 to 47.67 per cent, respectively of *F. oxysporum* f. sp. *lycopersici* in *in vitro* conditions.

Mailem *et al.* (2015) found that leaf extract of tulsi (5%) inhibited (63.70%) the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* in *in vitro* conditions.

2.4 Effect of different organic manure/cakes against *Fusarium oxysporum* f. sp. *ricini*

Haseeb and Kumar (2007) found that extract of farmyard manure and neem cake inhibits the growth of *Fusarium oxysporum* causing brinjal wilt in *in vitro* conditions.

Yelmame *et al.* (2010) studied eight organic manure/cakes against fusarium wilt of chilli in *in vitro* condition. Maximum inhibition (59.23%) of the growth of *Fusarium* was recorded in neem cake. Following mustard cake (52.61%), FYM (49.40%), groundnut cake (44.80%), poultry manure (42.29%), press mud (39.30%), castor cake (25.01%) and coconut cake (14.77%).

Patel and Patel (2012) reported that extract of farm yard manure, neem cake, Groundnut cake, Vermicompost and Castor cake at 10 per cent concentration inhibited the mycelial growth of *Fusarium udum* in *in vitro* condition by 5.93%, 14.81%, 9.63%, 2.22% and 11.85% respectively.

Mahalakshmi and Yesuraja (2013) reported that extract of neem cake, castor cake, coconut cake, groundnut cake, FYM, vermicompost and Poultry manure at 10 per cent concentration inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *dianthi* in *in vitro* conditions by 80.44%, 17.78%, 5.11%, 8.67%, 25.89%, 37.56% and 16.33% respectively.

Rani and Mane (2014) studied the effect of five organic amendment *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* by employing “Poison food technique”. Result revealed that highest per cent growth of inhibition was observed in soyabean cake 0.3 per cent (32.96%), followed by groundnut cake 0.3 per cent (29.63%), FYM 0.3 per cent (29.25%), Cotton cake 0.3 per cent (22.41%) and saw dust 0.3 per cent (16.85%).

2.5 Management of wilt under Pot condition.

Diyora and Khandar (1995) conducted a pot experiment to determine effect of oilcakes against *Fusarium oxysporum* f. sp. *cumini* and observed that the incidence of wilt was reduced in the treatments amended with organic cakes compared to control. The mortality of cumin plants was lowest (28.94%) in pots amended with mustard cake followed by groundnut cake (30.34%), castor cake (32.91%), neem cake (35.66%) and mahua cake (42.47%).

Goudar and Kulkarni (1998) conducted a pot experiment to determine the effect of organic amendments (groundnut cake, neem cake and farm yard) on the incidence of pigeon pea wilt caused by *Fusarium udum* and found that highest per cent survival of plants was recorded when neemcake was amended to the soil, followed by FYM and groundnut cake.

Madhukeshwara and Seshadri (2001) studied *in vitro* evolution of *Trichoderma viride*, *Pseudomonas fluoresces* and *Bacillus subtilis* against *Fusarium udum* by pot culture experiment. *Trichoderma viride* makes strong colony

inhibition, which was followed by *Pseudomonas fluorescens* and *Bacillus subtilis*.

Raju *et al.* (2005) conducted a pot experiment to determine the effect of *Trichoderma viride* on the incidence of pigeon pea wilt caused by *Fusarium udum* and found that combined use of *T. viride* + carbendazim + Rhizobium resulted in minimum wilt incidence (6.60%) followed by *T. viride* + carbendazim (11.10%).

Nikam *et al.* (2007) conduct a pot experiment to determine the effect of bioagent and oilseeds cakes against *Fusarium oxysporum* f. sp. *ciceri* in pot culture and result revealed that seed treatment of *Trichoderma viride* is found effective in controlling the chickpea wilt by (66.67%). The result obtained on the effect of different oilseeds cakes as a soil amendment proved to be effective in reducing disease under pot culture. Among oilseed cakes tested groundnut cake followed by neem seed.

Ram and Pandey (2011) studied *Trichoderma viride* and *P. fluorescens* reduced the incidence of *Fusarium udum* in *in vitro* conditions by pot culture experiment.

Athul *et al.* (2012) studied *In vitro* performance of the *Trichoderma* spp. and *Pseudomonas* spp., four effective antagonist isolate and two references strains were screened under pot culture for their biocontrol potential against the fungal pathogen *Fusarium oxysporum* with vanilla as a test plant and result revealed that *Trichoderma harzianum* and

Pseudomonas fluorescens were showing least percentage of leaves infection are (8.49%) and (7.18%) respectively.

Meghwal *et al.* (2014) studied *in vitro* evolution of *Trichoderma harzianum*, *Trichoderma viride*, *T. koningi* against *Fusarium oxysporum* f. sp. *ricini* by pot culture experiment. *T. harzianum* resulted minimum disease incidence of (4.61 %) and thereby reduced wilt of castor by (93.78%) followed by *T. viride* (88.60%) and *T. koningi* (86.19%).

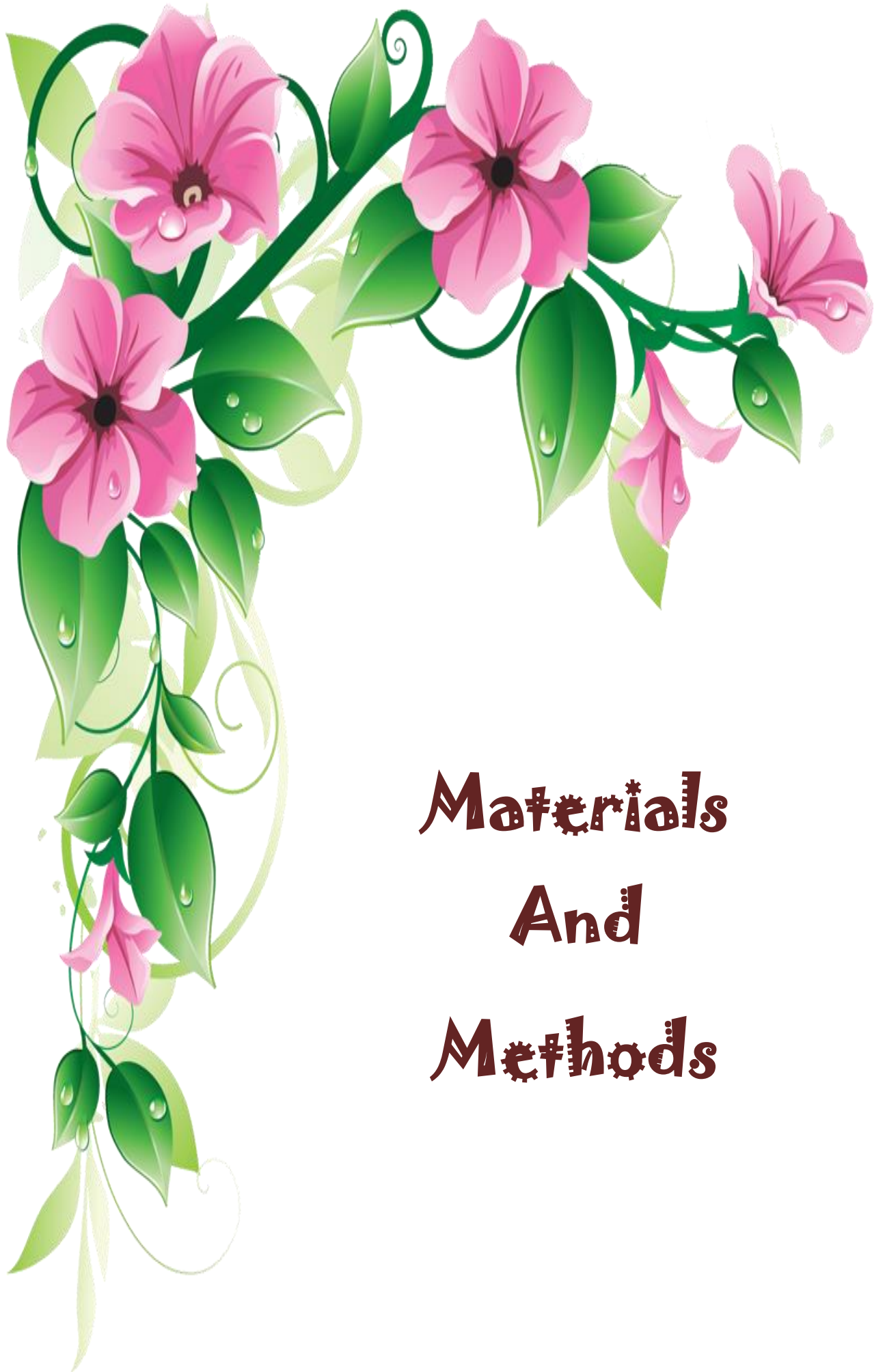
Magar *et al.* (2014) studied *in vitro* evolution of cotton cake, groundnut cake, *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *ciceri* by pot culture experiment. Found that among soil amendments cotton cake reduced wilt of chick pea by (43.41%) followed by groundnut cake (31.67%). Regarding bioagents, per cent disease control was recorded in *T. viride* (65.77%) followed by *T. harzianum* (64.67%).

Magar *et al.* (2014) conducted a pot experiment to determine the effect of oilcakes and bioagent against *Fusarium oxysporum* f. sp. *cicero* and result revealed that minimum wilt incidence was observed in cotton cake *i.e.* 48.93 per cent, while in groundnut cake 59.16 per cent wilt incidence was recorded. Regarding bioagents, minimum wilt incidence was recorded in *Trichoderma viride i.e.* 29.67 per cent followed by *Trichoderma harzianum* 35.60 per cent.

Kala *et al.* (2016) studied evolution of *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in pot culture. Found

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that *Pseudomonas fluorescens* resulted minimum disease incidence (51.83%) and reduced wilt of chickpea by (44.24%) which was followed by *T. harzianum* 41.63% and *T. viride* (37.97%).



Materials
And
Methods

III. MATERIALS AND METHODS

The details of the materials used and the methods adopted in the present investigation are described here as under.

GLASSWARES:

Different types of borosilicate glasswares viz., Petriplates, test tubes, conical flask, measuring cylinders, glass rods, slides, coverslips etc., used throughout study were cleaned by soaking in six percent chromic acid solution followed by washing with tepol and finally rinse with distilled water. These borosilicate glasswares were dried before use at room temperature.

STERILIZATION:

Glasswares

The borosilicate glasswares were sterilized in Hot air oven at 180°C for an hour.

Media

The liquid and solid media used throughout the study were sterilized by autoclaving at 1.2 kg / cm² for 15 minutes.

Soil

Soils used throughout the pot studies were sterilized twice by autoclaving at 1.2 kg/cm² for an hour each time.

Pots

The earthen pots of 30 cm diameter used were sterilized by dipping in two per cent formaldehyde solution for one minute.

INOCULATION AND INCUBATION:

In vitro

In *in vitro* studies, inoculations of Petriplates were carried out by 4 mm diameter mycelial discs, cut with the help of sterilized cork borer from the periphery of seven days old culture of *Fusarium oxysporum* f. sp. *ricini* from PDA. After inoculation, Petri plates were incubated at $27 \pm 2^{\circ}\text{C}$ for 7 days in oven or BOD incubator.

MULTIPLICATION OF INOCULUM FOR POT STUDY:

The inoculum of *F. oxysporum* f. sp. *ricini* was multiplied on sand maize meal medium by putting aseptically four mycelial discs of 4 mm diameter cut from the periphery of 7 days old pure culture grown on PDA. These inoculated flasks were incubated for 20 days and shaken at alternate day for uniform spread of the fungus growth. The inoculum of *F. oxysporum* f. sp. *ricini* was thoroughly mixed with the sterilized soil in the proportion of 1: 10 w/w and then filled in the pots. The pots were watered and kept for a week for uniform spread of the inoculum.

EXPERIMENTAL CONDITIONS:

The pot experiment was laid out near Biofertilizer unit, Navsari Agricultural University, Navsari. Laboratory

experiment was conducted in Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari.

3.1 PATHOLOGICAL INVESTIGATION:

3.1.1 Isolation and purification of the pathogen

Castor plants with typical wilt symptoms were collected from wilt affected field in Navsari district of Gujarat. The roots of such diseased plants were washed with running tap water to remove all adhered soil particles and they were subjected to tissue isolation.

Isolation of the pathogen from diseased specimen was made by tissue isolation technique. The typically infected roots and stem portions from the collar region were cut in to small pieces with the help of sterilized knife and again washed with the sterilized water. These pieces were then disinfected for one minute in 0.1 per cent mercuric chloride solution. To remove residue of mercuric chloride, the pieces were washed thrice in sterilized distilled water for one minute each time and pieces were then transferred aseptically on to potato dextrose agar (PDA) medium in Petri plates. The Petri plates were incubated for five days at $27 \pm 2^{\circ}\text{C}$ temperature in BOD incubator or Oven. The growth of pathogen obtained from the pieces was transferred on PDA slants and incubated for further growth at $27 \pm 2^{\circ}\text{C}$ temperature. These cultures were further purified by single spore isolation method. These pure culture isolates were maintained on PDA slants in refrigerator at $5 \pm 2^{\circ}\text{C}$ temperature. The streptomycin was added after autoclaving the media to avoid bacterial contamination.

3.1.2 Pathogenicity and Identification

In order to test the pathogenicity of pathogen collected from Navsari District of Gujarat, soil inoculation technique in pots was followed (Desai, 1999). Fungus inoculum multiplied on sand maize meal medium was thoroughly mixed with the autoclaved soil in the proportion of 1: 10 w/w, then filled in the pot, moistened with water and kept for a week for uniform spread of the inoculum. Surface sterilized 10 seeds of castor genotype GCH-4 (susceptible) were sown in pots. To prove the pathogenicity three pots were maintained. Pot filled with autoclaved, uninoculated soil and seeds sown at the same time served as control. The pots were watered with tap water as and when needed and left undisturbed for disease development. The observations on wilt incidence were recorded periodically up to 30 days. Reisolation was made from diseased seedlings of isolate and the cultures were compared with original cultures.

3.2 EFFICACY OF NATIVE BIO-AGENTS AGAINST *Fusarium oxysporum* f. sp. *ricini*

3.2.1 Collection and identification

Native isolates of bio-agents listed in (Table-1) were obtained from Department of Plant Pathology, N. M. C. A., Navsari and same were tested against *Fusarium oxysporum* f. sp. *ricini* by using dual culture technique.

3.2.2 *In vitro* evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ricini*

The test fungal BCA and pathogen were grown separately on PDA. From seven day old culture, 4 mm discs of the test fungal BCA and *F. oxysporum* f. sp. *ricini* were cut aseptically from the periphery of the colony and placed opposite to each other approximately 60 mm apart on to PDA contained in the Petri plates as adopted by Dennis and Webster (1971a).

Three repetitions of each treatment were kept and the Petri plates with 4 mm diameter discs of 7 days old culture of the pathogen on PDA served as control. The Petri plates were incubated at $27 \pm 2^{\circ}\text{C}$ temperature and after seven days of incubation, radial growth of the test organism and the *F. oxysporum* f. sp. *ricini* was measured. Per cent growth inhibition of the fungus in each treatment in comparison to control was calculated by the following equation (Bliss, 1934):

$$\text{PGI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

3.2.3 *In vitro* evaluation of *Bacillus* and *Pseudomonas* species against *Fusarium oxysporum* f. sp. *ricini*

To determine the antagonistic action of isolates of bacterial species viz., *Bacillus subtilis* and *Pseudomonas fluorescence* against *F. oxysporum* f. sp. *ricini* *in vitro*, 20 ml PDA medium was poured aseptically in each Petri plate and

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allowed to solidify. The bacterial antagonists were then streaked on one end of the Petri plate onto PDA medium 24 hrs. prior to *F. oxysporum* f. sp. *ricini* inoculation and just opposite to bacterial streak a 4 mm diameter disc of *F. oxysporum* f. sp. *ricini* from seven days old culture was placed.

Three repetitions of each treatment were kept and the Petri plates with 4 mm diameter discs of 7 days old culture of the pathogen on PDA served as control. The Petri plates were incubated at $27 \pm 2^{\circ}\text{C}$ temperature and after seven days of incubation the radial growth of the *F. oxysporum* f. sp. *ricini* was measured. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation given by Bliss, 1934 as mentioned earlier in 3.2.2.

Table-1: Antagonist screened against *Fusarium oxysporum* f. sp. *ricini* in vitro

Sr. no.	Treatment	Antagonistic
1	T ₁	<i>Trichoderma viride</i> Navsari isolate
2	T ₂	<i>Trichoderma harzianum</i> Navsari isolate
3	T ₃	<i>Trichoderma longibrachyatum</i> Navsari isolate
4	T ₄	<i>Trichoderma koningi</i> Navsari isolate
5	T ₅	<i>Trichoderma faciculatum</i> Navsari isolate
6	T ₆	<i>Pseudomonas fluorescens</i> Navsari isolate
7	T ₇	<i>Bacillus subtilis</i> Navsari isolate
8	T ₈	Control

3.3 Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*

3.3.1 Botanicals

Extract of medicinal plants tested against castor wilt pathogen *F. oxysporum* f. sp. *ricini* were, leaves of *Bougainvillea spectabilis* L., *Datura stramonium* L., *Ocimum sanctum* L., *Hibiscus bombycideron*, *Tegates erecta.*, rhizome of *Zingiber officinale* Rosc., *Curcuma longa* L. (Table 2). Solvents *i.e.* distilled water was employed for extraction from plant parts and results were derived based on comparative toxicity value.

Different parts of plants including leaf and rhizome tested were first washed with sterile distilled water, then sterilized with 90 per cent methanol and then air-dried. Weighed 100 g plant material was crushed in electrically operated mixer and grinder using 1:1 w/v amount of distilled water (Singh and Majumdar, 2001). The material was homogenized for 5 minutes and filtered through double layered muslin cloth and filtrate was centrifuged at 5000 rpm for 15 minutes and clear supernatant was collected. This was considered as 100 per cent concentration and used for experiment at various percentages.

3.3.2 *In vitro* evaluation

For evaluation of antifungal activities of the extract, desired concentrations (5%) were obtained by adding appropriate amount of standard solution of plant extracts (Sterilized) to 100 ml potato dextrose agar medium in conical

MATERIALS AND METHODS

flasks. Then about 20 ml extract mixed PDA was poured in sterilized Petri plates. After, solidification of PDA, plates was inoculated with 4 mm diameter discs of seven days old culture of *F. oxysporum* f. sp. *ricini* raised on PDA medium. Each treatment was repeated three times. PDA inoculated with 4 mm fungal discs without plant extract served as control. The inoculated plates were incubated at $27 \pm 2^{\circ}\text{C}$ for 7 days and diameter of colony was measured.

The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation given by Bliss, 1934 as mentioned earlier in 3.2.2.

Table-2: Botanicals tested against *Fusarium oxysporum* f. sp. *ricini* in vitro

Sr. No.	Treatment	Local name	Botanical name (5%)	Plant parts used for preparation of extracts
1	T ₁	Bougainvillea	<i>Bougainvillea spectabilis</i> L.	Leaves
2	T ₂	Ginger	<i>Zingiber officinalis</i> Rosa.	Rhizome
3	T ₃	Datura	<i>Datura stramonium</i> L.	Leaves
4	T ₄	Turmeric	<i>Curcuma longa</i> L.	Rhizome
5	T ₅	Tulsi	<i>Ocimum sanctum</i> L.	Leaves
6	T ₆	Jasud	<i>Hibiscus bombycideron</i>	Leaves
7	T ₇	Marigold	<i>Tegetes erecta</i>	Leaves
8	T ₈	Control	----	-----

3.4 Effect of different organic manure /cakes against *Fusarium oxysporum* f. sp. *ricini*

3.4.1 Methodology

The aqueous extract of different organic materials was prepared by suspending 30g of each organic material in 150 ml sterilized distilled water in flask and left for 25 days. The flasks were shaken on alternate day for thoroughly mixing and dissolution of the content. After 25 days, the flasks were thoroughly shaken and content was filtered through double layered muslin cloth and autoclaved at 1.2 kg cm² pressure for 20 minute. The sterilized extract was used for testing their inhibitory effect on *Fusarium oxysporum* f. sp. *ricini in vitro* by poisoned food technique. The autoclaved extract were individually added in previously sterilized melted and cooled potato dextrose agar medium @ 10 per cent v/v at the time of pouring in Petriplates and mixed thoroughly. All the plates were incubated at room temp. (27±2°C) after placing the 4 mm disc of actively growing 7 days old pure culture of *Fusarium oxysporum* f. sp. *ricini* for experiment were kept for each treatment. Medium without organic extract served as control.

Table-3: Organic manure/cakes screen against *Fusarium oxysporum* f. sp. *ricini in vitro*

Sr. No.	Treatment	Organic extracts
1	T ₁	Farm Yard Manure
2	T ₂	Neem cake
3	T ₃	Castor cake
4	T ₄	Groundnut cake
5	T ₅	Coconut cake
6	T ₆	Vermicompost
7	T ₇	Poultry manure
8	T ₈	Control

3.5 Management of wilt under Pot condition.

3.5.1 Evaluation of antagonists against castor wilt as seed treatment in pots

The antagonists which found promising *in vitro* study were tested for the control of castor wilt in pots as seed treatment.

The inoculum of *F. oxysporum* f. sp. *ricini* was multiplied on sand maize meal medium for 20 days at $27 \pm 2^\circ\text{C}$ and thoroughly mixed with the sterilized soil in proportion of 1:10 w/w, and then filled in the sterilized pots. The pots were watered and kept for a week for uniform spread of the pathogen. The formulation of each two promising bioagent@ 6g/kg seed was prepared. The seeds of castor variety were treated with the bioagent@ 6g/kg seed formulation. Seeds

sown without treatment in the inoculated pots served as control.

Two biocontrol agents @ 6g/kg treated with fifteen seeds of castor hybrid GCH-4 were sown in each pot and ten seeds were maintained. Untreated seeds were sown in inoculated pots served as control. Three repetitions were maintained for each treatment. They were uniformly irrigated soon after seed sowing. Observation on wilt incidence was recorded periodically up to 30 days after sowing and data were statistically analyzed.

3.5.2 Evaluation of different Botanicals against castor wilt as seed treatment in pots

Two Botanicals, which were found promising in *in vitro* study, were tested for the control of castor wilt in the pots. The inoculum of *F. oxysporum* f. sp. *ricini* multiplied on sand maize meal medium for 20 days at $27 \pm 2^{\circ}\text{C}$ was thoroughly mixed with the sterilized soil in proportion of 1 : 10 w /w and then filled in the sterilized pots. The pots were watered and kept for a week for uniform spread of the pathogen.

Fifteen seeds of castor hybrid GCH-4 were treated with 5 per cent concentration of respective botanicals and ten seeds were maintain and then dried at room temperature. Seeds treated with distilled water served as control. Seeds treated with two different plant extracts were sown in *F. oxysporum* f. sp. *ricini* inoculated pots (10 seeds / pot) along with control treatment and three repetitions for each treatment were kept.

Pots arranged for wilt development and irrigated as and when required. Observation on wilt incidence was taken up to 30 days after sowing.

3.5.3 Evaluation of different organic/cakes against castor wilt as seed treatment in pots

Two organic, which were found promising in *in vitro* study, were tested for the control of castor wilt in the pots. The inoculum of *F. oxysporum* f. sp. *ricini* multiplied on sand maize meal medium for 20 days at $27 \pm 2^{\circ}\text{C}$ was thoroughly mixed with the sterilized soil in proportion of 1 : 10 w /w and then filled in the sterilized pots. The pots were watered and kept for a week for uniform spread of the pathogen.

Fifteen seeds of castor hybrid GCH-4 were treated with 5 per cent concentration of respective organic extracts and ten seeds were maintained and then dried at room temperature. Seeds treated with distilled water served as control. Seeds treated with two different plant extracts were sown in *F. oxysporum* f. sp. *ricini* inoculated pots (10 seeds / pot) along with control treatment and three repetitions for each treatment were kept. Pots arranged for wilt development and irrigated as and when required. Observation on wilt incidence was taken up to 30 days after sowing.

Experimental details:

MATERIALS AND METHODS

Experimental design	CRD
Number of treatments	7
Number of repetition	3
Crop/ Variety	Castor/ GCH-4
Number of plants/pot	10
Method	Seed treatment

Observation to be recorded:

Per cent disease incidence = $\frac{\text{Number of infected plant} \times 100}{\text{Total no. of plant}}$

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

Where,

I = Per cent disease control

C = Per cent disease incidence in control

T = Per cent disease incidence in treatment



Results
And
Discussion



Plate-1 : Field view of wilt of castor

IV. RESULTS AND DISCUSSION

4.1 Diagnosis of disease

4.1.1 Collection of specimen

Castor plants (GCH-4) showing the typical symptoms were collected from Mega Seed, Pulses and Castor Research Unit, NAU, Navsari as well as from farmer's field and brought to the laboratory and subjected to tissue isolation.

4.1.2 Isolation and purification of pathogen

Castor plants with typical wilt symptoms were collected from Mega Seed, Pulses and Castor Research Unit and farmer's field and subjected to isolation by tissue isolation technique. These cultures were further purified by single spore isolation method. Pure cultures were maintained on PDA slants and used for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the investigations.

4.1.3 Pathogenicity and Identification

The pathogenicity test of the *Fusarium oxysporum* f. sp. *ricini* established from wilted castor plants, collected from different Navsari District of Gujarat state was tried by soil inoculation technique on castor genotype GCH-4 (susceptible) in pots.



Plate-2 : Pure culture of *Fusarium oxysporum* f. sp. *ricini*

A. Mycelium



B. Spore

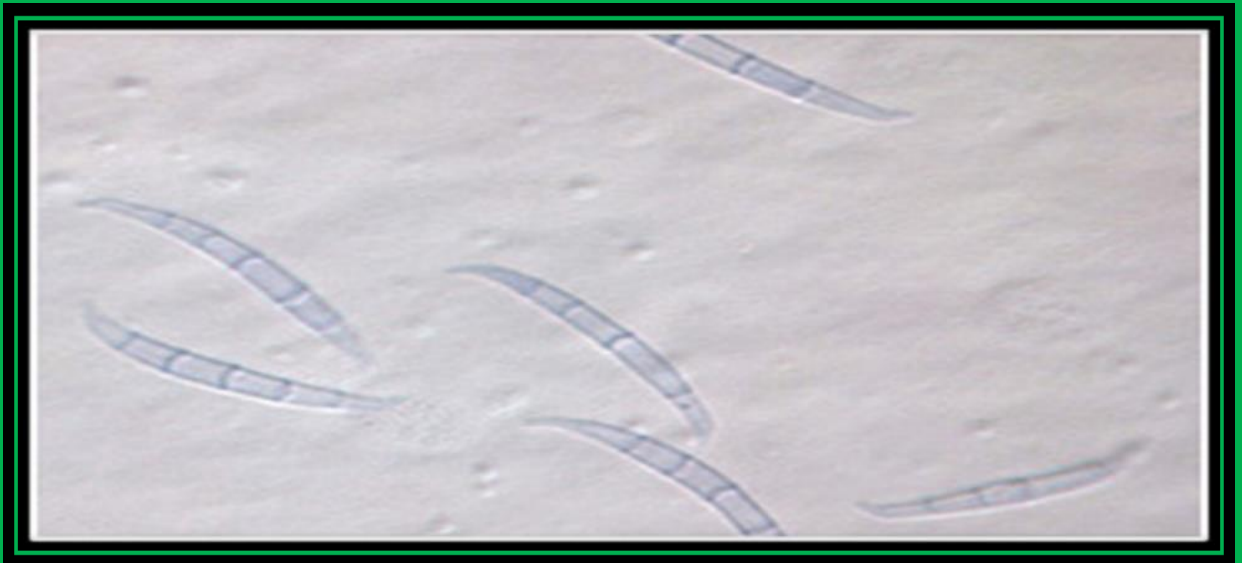


Plate-3 : Microphotograph of mycelium and spore of *Fusarium oxysporum* f. sp. *ricini*

RESULTS AND DISCUSSION

The visible wilt symptoms initially appeared after 12-16 days of sowing in pot in which pathogen were mixed in the soil. Symptoms manifested were as yellowing, marginal and inter-veinal necrosis of leaves, which later on the plants withered and finally dried. The plants of uninoculated control pots did not show any wilt symptoms and remained completely healthy.

Freshly wilted plants were collected from the pots inoculated with pathogen and reisolations were made from the lower stem portions of wilted plants, separately. These cultures were individually compared with original cultures. Pathogen produced wilt incidence in castor plants by soil inoculation technique in pot study and yielded pure cultures in reisolation, which resembled with original cultures of *F. oxysporum* f. sp. *ricini* on the basis of their cultural and morphological characteristics.

Nanda and Prasad (1974) proved the pathogenicity of the wilt pathogen of castor with soil inoculation technique and causal organism was identified as *F. oxysporum* f. sp. *ricini*. In the present study, pathogen collected from Navsari District of Gujarat state were found to be pathogenic with wilt



Healthy plant

Control



Wilted plant

Inoculated

Plate-4 : Pathogenicity test for *Fusarium oxysporum* f. sp. *ricini*

incidence on castor hybrid GCH-4 and resembled with *F. oxysporum* f. sp. *ricini* on the basis of their cultural, morphological characteristics and pathogenicity test.

4.2. Efficacy of bioagent against *Fusarium oxysporum* f. sp. *ricini*

The hazardous effect of chemical used in plant disease management has diverted plant pathologist to find out alternative methods having little or no adverse effect on environment. Notable success of disease control through the use of antagonists microorganism in the laboratory, glass house and field have been achieved during past several years and based on this information, there is a possibility of developing biological control of plant disease under field conditions. Now a days the commercial formulation of some of the bio-control agents are already available in the market. However, inadequate information on the performance of the antagonists under varying condition is a major constraint in the large scale adoption of this technology.

In this study, pure culture of *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma longibrachyatum*, *Trichoderma koningii*, *Trichoderma faciculatum*, *Pseudomonas fluorescence* and *Bacillus subtilis* obtained from Department of Plant Pathology, N. M. College of Agriculture, N.A.U., Navsari, were tested *in vitro* by dual culture method to find out the antagonistic effect.

4.2.1 Dual culture technique

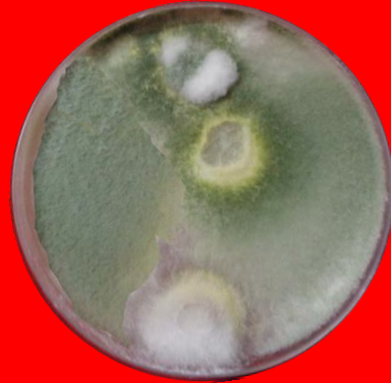
Table-4: Efficacy of native bio-agents against *Fusarium oxysporum* f. sp. *ricini* in vitro

Sr. No.	Bioagents	Colony diameter of pathogen (mm)	Growth inhibition over control (%)
1	<i>Trichoderma viride</i> Navsari isolate	26.67	70.37 (57.03)
2	<i>Trichoderma harzianum</i> Navsari isolate	25.00	72.22 (58.19)
3	<i>Trichoderma longibrachyatum</i> Navsari isolate	32.00	64.44 (53.41)
4	<i>Trichoderma koningii</i> Navsari isolate	33.33	62.96 (52.51)
5	<i>Trichoderma faciculatum</i> Navsari isolate	40.33	55.19 (47.98)
6	<i>Pseudomonas fluorescens</i> Navsari isolate	29.00	67.78 (55.42)
7	<i>Bacillus subtilis</i> Navsari isolate	49.33	45.19 (42.24)
8	Control	90.00	0.00 (0.59)
	S.Em.±	0.99	
	C.D. at 5%	2.96	
	C.V.%	4.20	

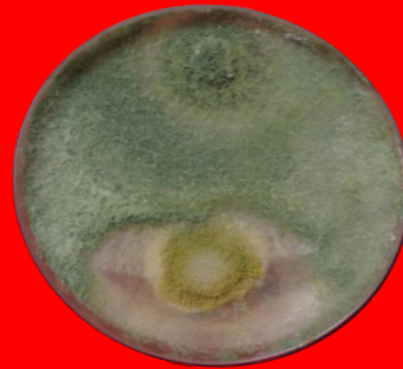
*Figures in parenthesis are Angular transformation value



T. viride



T. harzianum



T. longibrachyatum



T. koningii



T. faciculatum



P. fluorescens



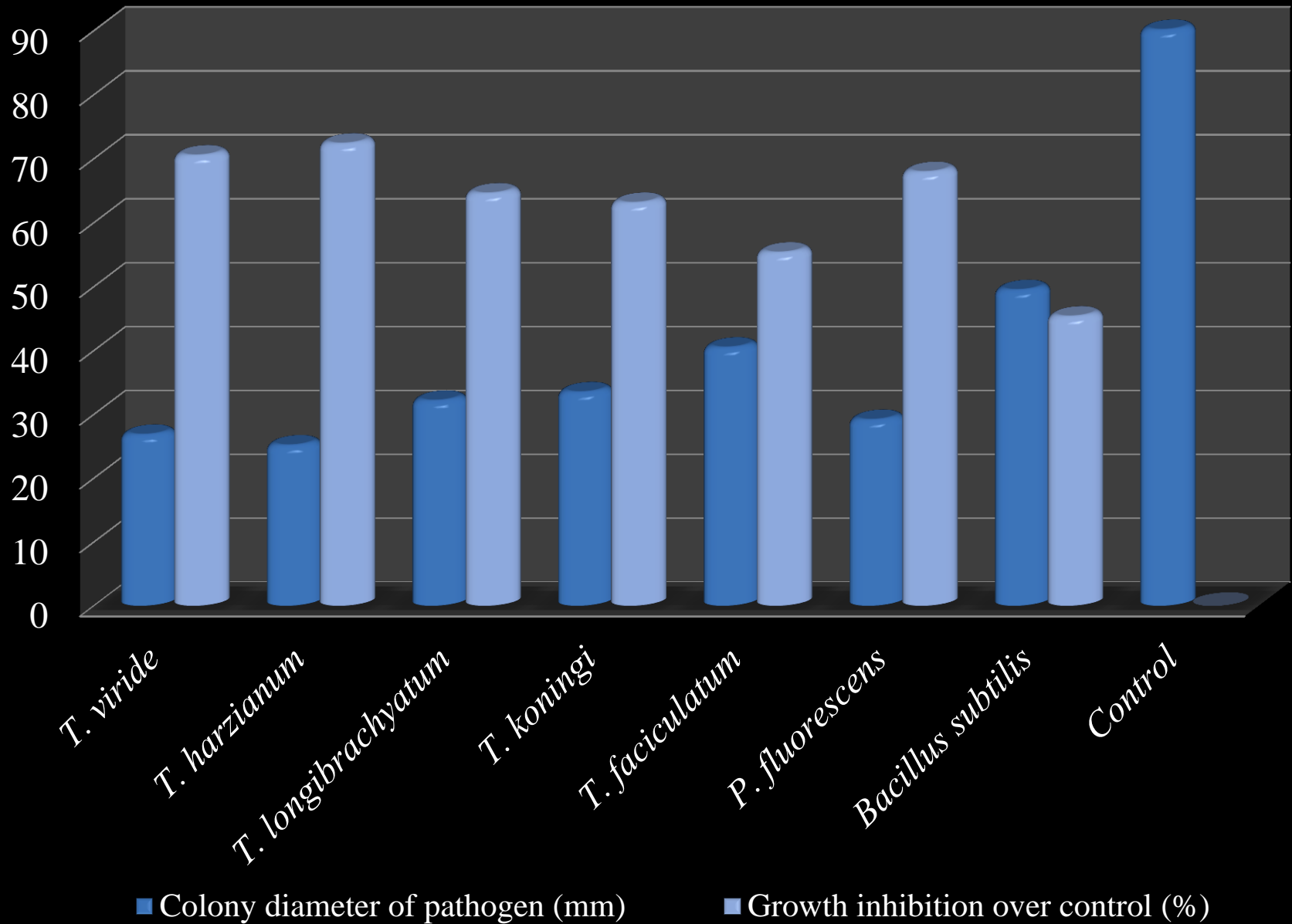
Bacillus subtilis



Control

Plate-5 : Efficacy of native bioagent against *Fusarium oxysporum* f. sp. *ricini*

Fig.-1: Efficacy of native bio-agents against *Fusarium oxysporum* f. sp. ricini



RESULTS AND DISCUSSION

The result presented in table-4, [Plate-5] and depicted graphically in Fig.-1 revealed that, all the antagonists tested against *Fusarium oxysporum* f. sp. *ricini* were effective in checking the growth of the pathogen. Out of seven antagonists tested, least growth of the pathogen was recorded in *Trichoderma harzianum* (25.00 mm) which was at par with *Trichoderma viride* (26.67 mm) followed by *Pseudomonas fluorescens* (29.00 mm), *Trichoderma longibrachytum* (32.00 mm), *Trichoderma koningi* (33.33 mm), *Trichoderma faciculatum* (40.33 mm) and *Bacillus subtilis* (49.33 mm).

The result presented in Table-4 revealed that, *Trichoderma harzianum* (72.22%), *Trichoderma viride* (70.37%), *Pseudomonas fluorescens* (67.78%), *Trichoderma longibrachytum* (64.44%) and *Trichoderma koningi* (62.96%) significantly inhibited the pathogen. Whereas, *Trichoderma faciculatum* (55.19%) and *Bacillus subtilis* (45.19%) were comparatively least effective.

It is evident from these studies that among all the antagonists evaluated by dual culture method, *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* consistently showed strong antagonistic property against *Fusarium oxysporum* f. sp. *ricini* compared to the other antagonists tested hence considered as potential antagonists. Our results are in harmony with earlier workers Meghwal *et al.* (2014) found that *T. harzianum* and *T. viride* significantly inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *ricini* (68-72%) by Dual culture technique. Patil *et al.* (2015) carried out interaction study of known antagonist by dual

culture method and found strong antagonistic effect on *Fusarium oxysporum* f. sp. *ciceri* with *T. viride*, *T. koningii*, *G. virens*, *T. pseudokoningii* and *Pseudomonas fluorescens* *in vitro*.

4.3 Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*

Many botanicals are known to have inhibitory effect on the growth and reproduction of various fungi. This information is certainly useful in exploiting inhibitory principles for developing botanical fungicides for plant disease management. In the present investigation, 7 botanicals of different plant species prepared in solvent fractions (water) with suitable control were screened by poisoned food technique *in vitro* to know their inhibitory effect on the growth of the *F. oxysporum* f. sp. *ricini*.

The result presented in table-5, [Plate-6] and depicted graphically in Fig.-2 sterilized revealed that all the plant extract inhibited the growth of the fungus as compared to control.

The Turmeric extract (*Curcuma longa* L.) 49.00 mm allowed minimum growth of the pathogen followed by Marigold (*Tegetes erecta* L.) 54.00 mm, Tulsi (*Ocimum sanctum* L.) 56.00 mm, Bougainvillea (*Bougainvillea spectabilis* L.) 58.33 mm, Jasud (*Hibiscus bombycideron* L.) 59.33 mm, Ginger (*Zingiber officinalis* Rosa.) 63.67 mm and Datura (*Datura stramonium* L.) 70.00 mm in sterilized plant extract.

Table-5: Efficacy of botanicals against *Fusarium oxysporum* f. *sp. ricini in vitro*

Sr. No.	Name of plant	Botanical name (5%)	Plant parts used for preparation of extract	Average colony diameter of pathogen (mm)	Growth inhibition over control (%)
1	Bougainvillea	<i>Bougainvillea spectabilis</i> L.	Leaves	58.33	31.85 (35.02)
2	Ginger	<i>Zingiber officinalis</i> Rosa.	Rhizome	63.67	25.92 (31.19)
3	Datura	<i>Datura stramonium</i> L.	Leaves	70.00	18.89 (26.23)
4	Turmeric	<i>Curcuma longa</i> L.	Rhizome	49.00	42.22 (41.36)
5	Tulsi	<i>Ocimum sanctum</i> L.	Leaves	56.00	34.44 (36.64)
6	Jasud	<i>Hibiscus bombycideron</i>	Leaves	59.33	30.74 (34.28)
7	Marigold	<i>Tegetes erecta</i>	Leaves	54.00	36.67 (38.01)
8	Control	87.00	0.00 (0.58)
	S.Em.±			1.32	
	C.D. at 5%			3.97	
	C.V.%			3.69	

*Figures in parenthesis are Angular transformation value



Bougainvillea



Ginger



Datura



Turmeric



Tulsi



Jasud



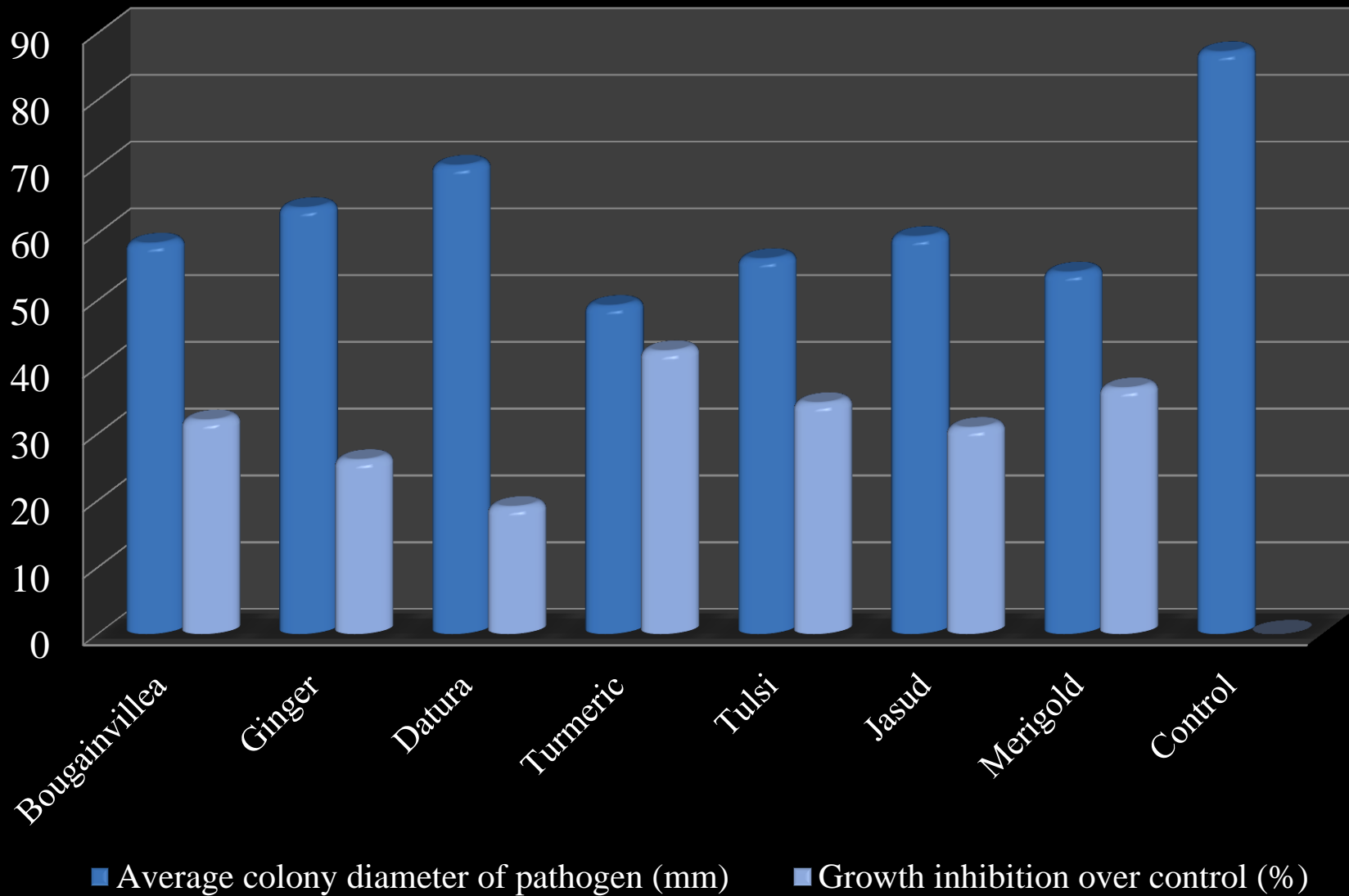
Merigold



Control

Plate-6: Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*

Fig.-2 Efficacy of botanicals against *Fusarium oxysporum* f. sp. *ricini*



The Turmeric rhizome extract (*Curcuma longa* L.) 42.22 per cent showed maximum growth inhibition of the pathogen followed by marigold leaves (*Tegetes erecta* L.) 36.67 per cent, Tulsi leaves (*Ocimum sanctum* L.) 34.44 per cent, Bougainvillea leaves (*Bougainvillea spectabilis* L.) 31.85 per cent, Jasud leaves (*Hibiscus bombycideron* L.) 30.74 per cent, Ginger (*Zingiber officinalis* Rosa.) 25.93 per cent and Datura (*Datura stramonium* L.) 18.89 per cent in sterilized plant extract.

The present studies are in confirmation with those described by earlier worker Chohan and Perveen (2014) studied the effect of botanicals on *Fusarium oxysporum* f. sp. *lycopersici* and reported that out of rhizomes extracts tested, Turmeric (*Curcuma longa* L.) extract was found most inhibitory to *Fusarium oxysporum* f. sp. *lycopersici*. Singh *et al.* (2014) reported fungitoxic properties of Turmeric, Datura, Ginger, Tulsi and marigold and against growth and of *Fusarium oxysporum* f. sp. *lini*. Shalini *et al.* (2015) tested botanicals, among them turmeric extracts (5%) gave 68.05 per cent and ginger extract (5%) gave 45.92 per cent inhibition of *Fusarium oxysporum* f. sp. *ricini*.

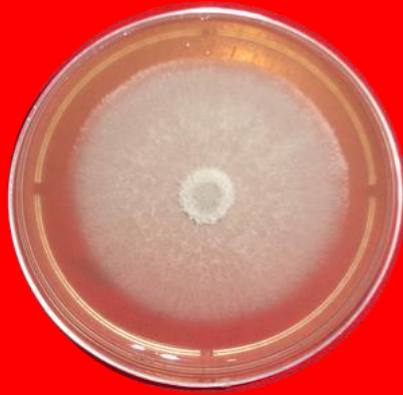
4.4. Effect of different organic manure/cakes against *Fusarium oxysporum* f. sp. *ricini*

The aqueous extract of different organic were evaluated for their inhibitory effect on *Fusarium oxysporum* f. sp. *ricini*. The result presented in Table-6 [Plate-7] and depicted graphically in Fig.-3 indicated that organic extract product

Table-6: Effect of different organic manure/cakes against *Fusarium oxysporum* f. sp. *ricini* in vitro

Sr. No.	Name of extract	Average colony diameter of pathogen (mm)	Growth inhibition over control (%)
1	Farm Yard Manure	63.33	29.62 (32.93)
2	Neem cake	44.00	51.11 (45.64)
3	Castor cake	53.33	40.74 (39.66)
4	Groundnut cake	74.33	17.41 (24.66)
5	Coconut cake	51.33	42.96 (40.95)
6	Vermicompost	57.67	35.93 (36.82)
7	Poultry manure	46.67	48.15 (43.94)
8	Control	90.00	0.00 (0.58)
	S.Em.±	1.62	
	C.D. at 5%	4.86	
	C.V.%	4.67	

*Figures in parenthesis are Angular transformation value



FYM



Neem cake



Castor cake



Groundnut cake



Coconut cake



Vermicompost



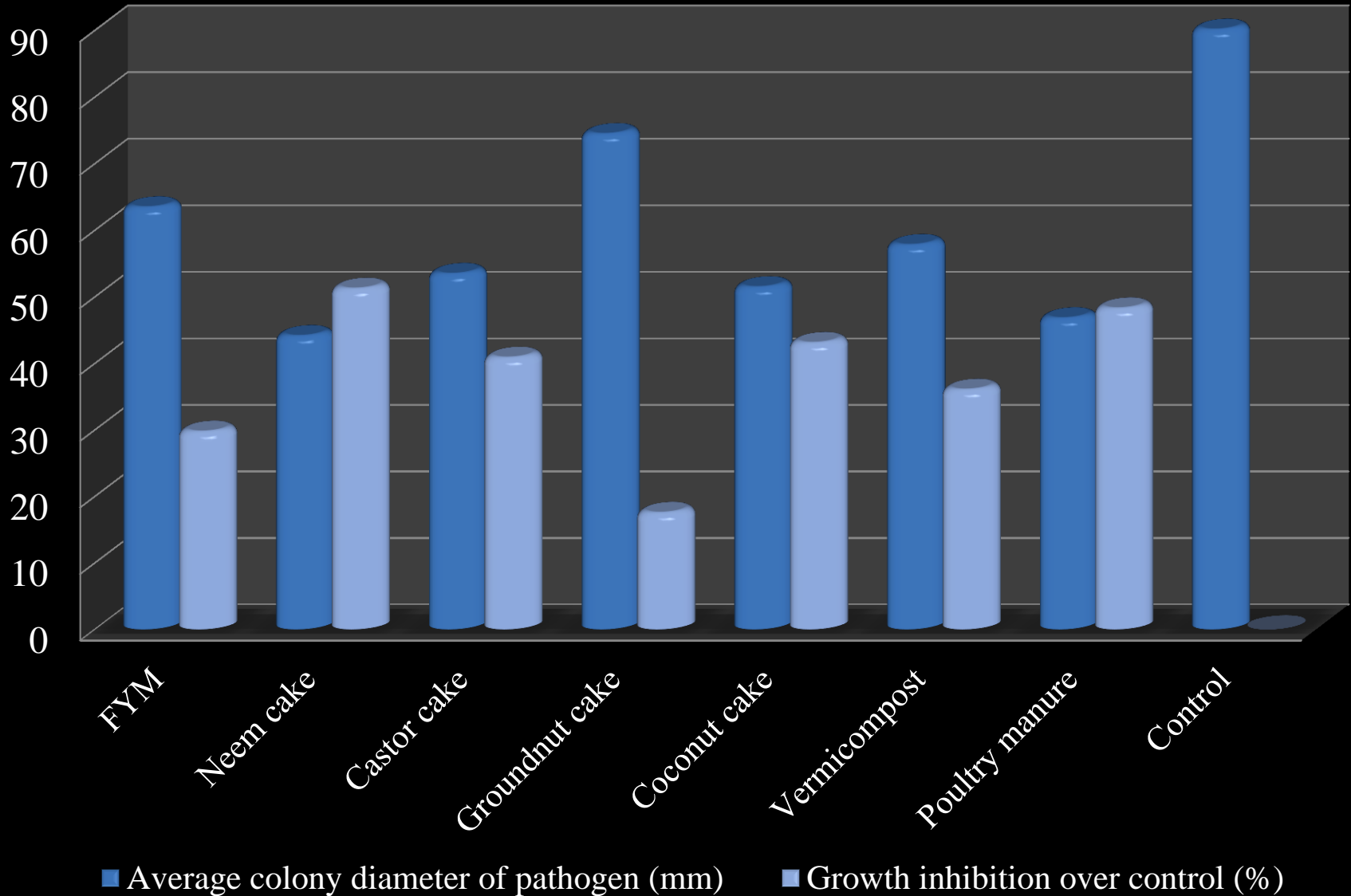
Poultry manure



Control

Plate-7 : Effect of different organic manure/cakes against *Fusarium oxysporum* f. sp. ricini

Fig.-3 Efficacy of different organic/cakes against *Fusarium oxysporum* f. *sp. ricini*



RESULTS AND DISCUSSION

significant inhibitory effect on the fungal growth. Among all the organic extract, minimum growth was recorded in the extract of neem cake (44.00 mm) followed by Poultry manure (46.67 mm), Coconut cake (51.33 mm), Castor cake (53.33 mm). Whereas, Vermicompost (57.66 mm), FYM (63.33 mm) and Groundnut cake (74.33 mm) were poor in inhibiting growth of the pathogen.

Maximum per cent growth inhibition of *Fusarium oxysporum* f. sp. *ricini* was recorded in neem cake (51.11%) followed by Poultry manure (48.15%), Coconut cake (42.96%), Castor cake (40.74%). Whereas, Vermicompost (35.93%), FYM (29.63%) and Groundnut cake (17.41%) were least effective in inhibiting the growth of *Fusarium oxysporum* f. sp. *ricini*.

From this study, it is clear that neem cake and Poultry manure were found effective in reducing the growth of *Fusarium oxysporum* f. sp. *ricini* causing wilt in castor. The present investigation is more or less similar to the work done by earlier worker Yelmame *et al.* (2010) reported that extracts of neem cake inhibited the growth of *Fusarium oxysporum* f. sp. *capsici* by 59.23 per cent in laboratory condition. Effect of neem cake, castor cake, coconut cake, groundnut cake. FYM, vermicompost and poultry manure @ 10 per cent recorded inhibit the mycelial growth (80.44%), (17.78%), (5.11%), (8.67%), (25.89%), (37.56%) and (16.33%), respectively against *Fusarium oxysporum* f. sp. *dianthii* (Mahalakshmi and Yesuraja, 2013).

4.5. Management of wilt under Pot condition

After the world wide stimulation of interest in biocontrol of plant disease during the past decade, attempts to develop bio-control method for the management of wilt disease were made by several workers.

The effective biocontrol agents, botanicals and organic manures/cakes which were found promising under laboratory studies were further evaluated for the management of wilt (*Fusarium oxysporum* f. sp. *ricini*) disease of castor under pot conditions. The antagonist (*Trichoderma harzianum* and *Trichoderma viride*), botanicals (Turmeric and marigold) and organic (Neem cake and Poultry manure) applied as a seed dresser, were evaluated for their efficacy against wilt pathogen under pot condition.

The data presented in table-7 and Fig.-4 revealed that the effects of all the treatments were found significantly superior over control in managing the wilt disease of castor.

Considering disease incident in seed treatment (*Trichoderma harzianum* @ 6g/kg seed) has lowest (23.33%) disease incidence followed by seed treatment with (*Trichoderma viride* @ 6g/kg seed) (26.67%), seed treatment with turmeric extract (33.33%), seed treatment with marigold extract (36.67%), seed treatment with Neem cake (43.33%) and seed treatment with poultry manure (46.67%) disease incidence.

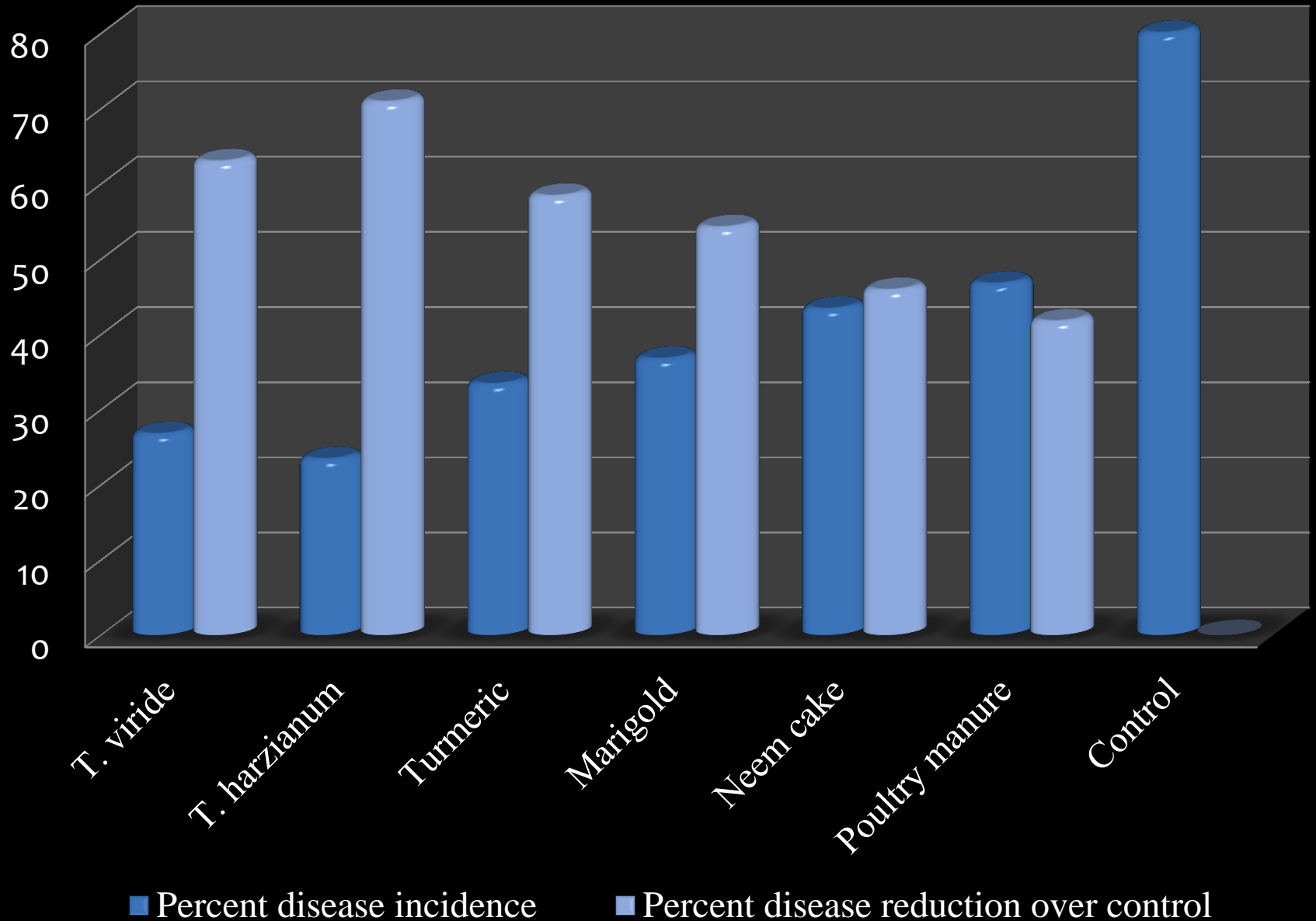
The similar results to our present investigation were achieved by Magar *et al.* (2014) reported that coconut cake,

Table-7: Management of wilt under pot condition

Sr. No.	Treatment	Average no. of plant infected	Percent disease incidence	Percent disease reduction over control
1	<i>Trichoderma viride</i> Navsari isolate	2.67	26.67 (35.17)	62.92 (54.83)
2	<i>Trichoderma harzianum</i> Navsari isolate	2.33	23.33 (32.59)	70.83 (57.41)
3	Turmeric	3.33	33.33 (40.17)	58.33 (49.83)
4	Marigold	3.67	36.67 (42.59)	54.17 (47.41)
5	Neem cake	4.33	43.33 (47.41)	45.83 (42.59)
6	Poultry manure	4.67	46.67 (49.83)	41.67 (40.17)
7	Control	8.00	80.00 (0.63)	0.00 (0.63)
	S.Em.±	0.31		
	C.D. at 5%	0.94		
	C.V. %	12.90		

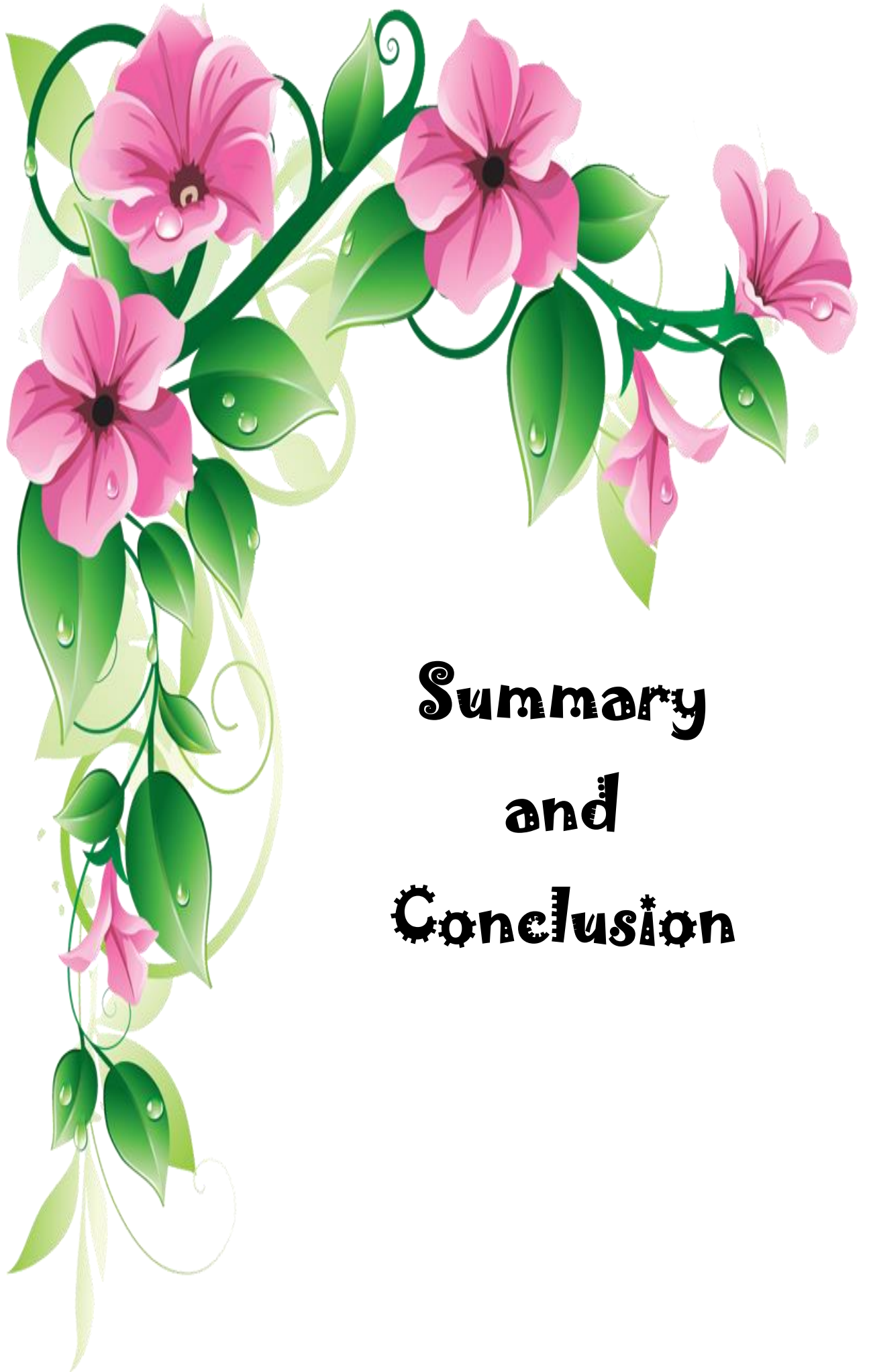
*Figures in parenthesis are Angular transformation value

Fig.-4 Management of wilt under Pot condition



RESULTS AND DISCUSSION

groundnut cake, *Trichoderma viride* and *Trichoderma harzinaum* were reduced wilt of check pea by (43.41%), (31.67%), (65.77%) and (65.77%) respectively. Meghwal *et al.* (2014) conducted to assess soil amendments and bioagent. *Trichoderma harzianum* and *T. viride* found most effective in reducing the disease of castor wilt caused by *Fusarium oxysporum* f. sp. *ricini*.



**Summary
and
Conclusion**

V. SUMMARY AND CONCLUSION

Castor (*Ricinus communis* L.) belonging to the *Euphorbiaceae* family is the most important non-edible oilseed crop of arid and semi-arid regions of India. In Gujarat, castor crop is cultivated in Banaskantha, Sabarkantha, Mehsana, Patan, Vadodara, Bharuch, Navsari, Valsad, Gandhinagar, Kheda, Panchmahal, Jamnagar, Kutchh, Rajkot and Junagadh districts. Wilt of castor caused by *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad is a serious problem in Gujarat state and causes heavy yield losses. Considering the fact, the present investigation was carried out on various aspects to generate more scientific information on the pathological problem, which include cause of the disease, identification of the causal organism, control through bioagent, phytoextract and organic extract *in vitro* and pot condition. The results obtained were depicted in previous chapter no. IV and were summarized and concluded in this chapter.

The fungus associated with the wilt disease in castor was isolated by tissue segment method from root region of wilt infected castor plants. It was identified as *Fusarium oxysporum* f. sp. *ricini* based on morphological and cultural characteristics.

The pathogenicity test carried out by soil inoculation method. Soil inoculation method successfully produced wilt symptoms similar to those under natural conditions as well as described in the literature, confirming pathogenic nature of the

fungus. The causal agent of castor wilt was identified and confirmed as *Fusarium oxysporum* f. sp. *ricini*.

All the *Trichoderma* species evaluated by dual culture method against *F. oxysporum* f. sp. *ricini* inhibited the growth of *F. oxysporum* f. sp. *ricini* by 45.19 to 72.22 per cent. *Trichoderma harzianum* showed maximum growth inhibition (72.22 %) of the pathogen after seven days of incubation and found superior among all the test organisms. The least growth inhibition (55.19 %) was recorded in *T. faciculatum*. Among bacterial species, *Pseudomonas flurorescens* showed maximum growth inhibition (67.18%) of the pathogen after seven days of incubation and found superior among bacterial antagonist in dual culture method. The least inhibition (45.19%) was observed by *Bacillus subtilis*.

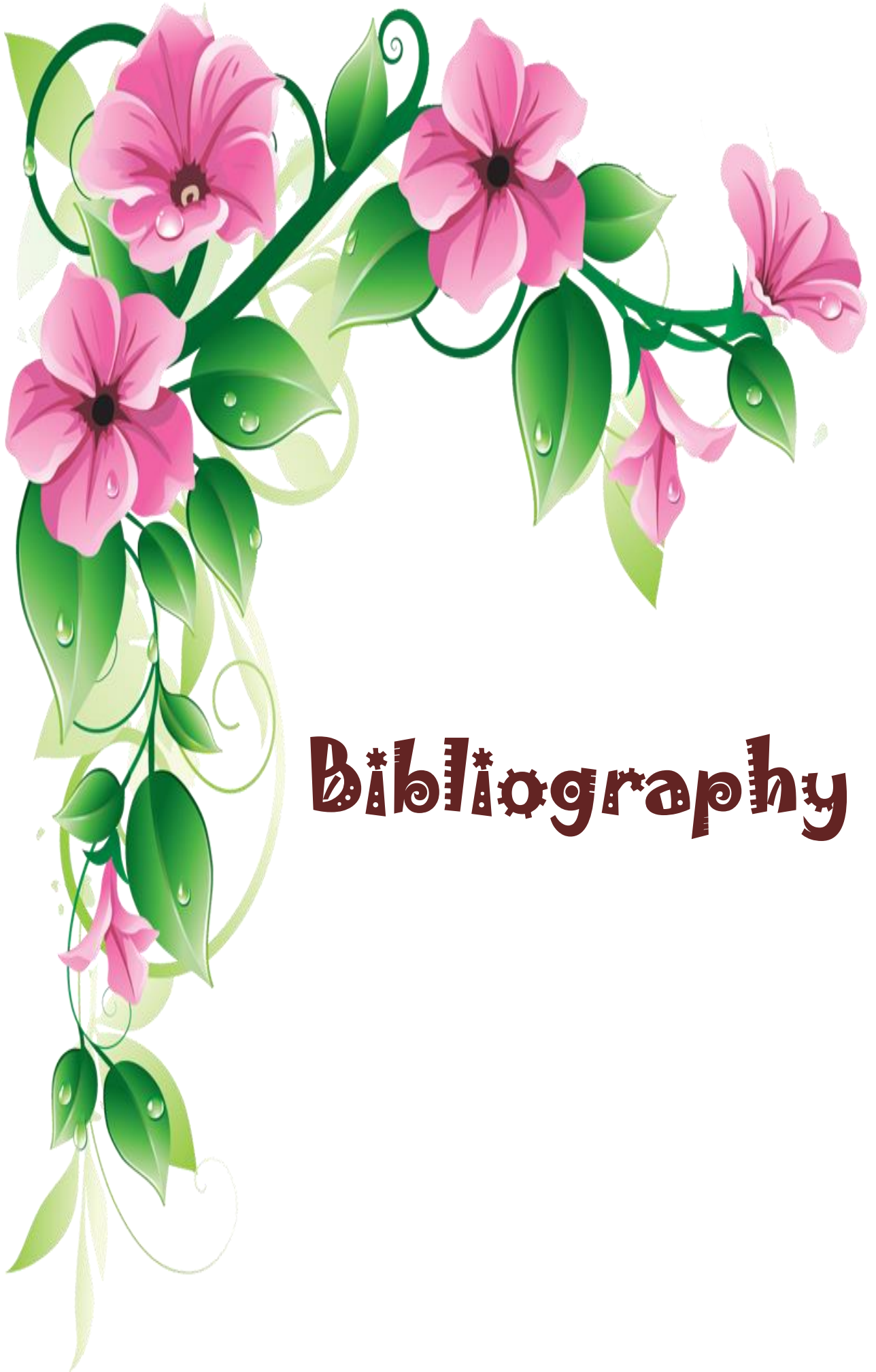
The phytoextract of commonly available seven different sterilized plant extract were tested in laboratory condition by poison food technique against *Fusarium oxysporum* f. sp. *ricini*. The extract of turmeric (*Curcuma longa* L.) was proved excellent in inhibiting mycelial growth (42.22%) followed by marigold (*Tegetes erecta* L.) 36.67 per cent. The next best is Tulsi (*Ocimum sanctum* L.), Bougainvillea (*Bougainvillea spectabilis* L.), Jasud (*Hibiscus bombycideron* L.), ginger (*Zingiber officinalis* Rosa.), Datura (*Datura stramonium* L.).

Seven different organic extracts were tested against *Fusarium oxysporum* f. sp. *ricini* by poisoned food technique with 5% concentration *in vitro*. All the extracts were

SUMMARY AND CONCLUSION

inhibitory to *Fusarium oxysporum* f. sp. *ricini* significantly lower mycelium growth was recorded in neem cake (51.11%) followed by Poultry manure (48.15%). Next best were coconut cake, castor cake and vermicompost. While lowest inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ricini* was observed in FYM and groundnut cake.

Pot experiment for management of wilt, indicate that seed treatment of (*Trichoderma harzianum* @ 6g/kg) has lowest disease incidence (23.33%) followed by seed treatment with (*Trichoderma viride* @6g/kg), Turmeric, Marigold, Neem cake and Poultry manure.



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