EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*

THESIS

Submitted To
Dr. Panjabrao Deshmukh Krishi Vidyapeeth,Akola
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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Dpachpute

Place : Akola **(PACHPUTE DEEPASHRI PRAKASH)**

Date : 12/06/2014 **Enrolment No : KK-2524**

CERTIFICATE

This is to certify that thesis entitled **"EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*"** submitted in partial fulfillment of the requirements for the degree of **"Master of Science in Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **PACHPUTE DEEPASHRI PRAKASH** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

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Date: 12/06/2014

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%	–	Per cent
/	–	Per
^{0}C	–	Degree celcius
C.D.	–	Critical difference
cm	–	Centimetre
e.g.	–	Exampli gratia (For example)
et al.	–	Et alia (and others)
etc.	–	Et cetra
f. sp.	–	forma species
Fig.	–	Figure
g	–	Gram
h	–	Hours
ha	–	Hector
i.e.	–	That is
J.	–	Journal
ml	–	milliliter
mm	–	millimeter
NA	–	Nutrient Agar
µl	–	micro liter
PDA	–	Potato dextrose agar
SE (m) $\pm$	–	Standard error of mean
Sig.	–	Significant
spp.	–	Species
viz,.	–	Videlicet (Namely)

(F) **THESIS ABSTRACT**

a) Title of the thesis	:	**" EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*"**
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ABSTRACT

Studies entitiled "Effect of herbicides on wilt of pigeonpea caused by *Fusarium udum*" was carried out during *kharif*, 2013-14 on the field of Pulses Research Unit and Laboratory of Department of Plant Pathology,Dr.Panjabrao Deshmukh Krishi Vidyapeeth,Akola.

The effect of seed dressing fungicides (thiram and carbendazim) and herbicides i.e pendimethalin and imazethapyr alone and combinations were tested against *Fusarium udum*, under different temperatures and pH ranges.

All the combinations of fungicides and herbicides recorded 100 per cent inhibition of the pathogen at all four pH level of 5.5, 6.5, 7.5, and 8.5 and temperature regimes of 20, 25 , 30 and 35^{0}C, indicating the harmful effect of herbicides on the *F. udum*.

In vitro study revealed 100 per cent mycelial growth inhibition of *F. udum* with Thiram @ 0.2 % + Carbendazim @0.1 % at all the temperature and pH studied. The pre-emergence herbicides *viz,* Pendimethalin @100 µl/100 ml medium (1.0 kg a.i./ha(PE)) showed 50.37 to 55.55 per cent and Imazethapyr @ 75 µl/100 ml (0.75 kg a.i./ha) showed 33.33 to 38.53 per cent inhibition; whereas, in combination both the herbicides recorded 17.77 to 27.0 per cent inhibition at various pH and at 20^{0}C tempearture.

At 25^{0}C temperature, pre-emergence herbicide Pendimethalin @100 µl/100 ml medium showed 43.94 to 64.80 per cent and Imazethapyr @ 75 µl/100 ml showed 80.21 to81.58 per cent inhibition of *F.* udum and in combination caused 32.92 to 41.37 per cent at various pH, when observed at 7th DAI.

The inhibition due to Pendimethalin @100 µl/100 ml medium was 61.18 to 62.84 per cent and due to Imazethapyr @ 75 µl/100 ml was by 70.35 to 83.19 per cent at various pH and 30^{0}C temperature. In combination both the herbicides pathogens growth was inhibited to the extent of 55.18 to 60.00 per cent at 7th DAI.

Similarly, at 35^{0}C effect of Pendimethalin@100 µl/100 ml medium exhibited 62.88 to 68.42 per cent inhibition at 5.5, 6.5, 7.5, and 8.5 pH, while Imazethapyr @ 75 µl/100 ml showed only 2.46 to 3.15 per cent inhibition of *F. udum.* Pendimethalin @100 µl/100 ml medium + Imazethapyr @ 75 µl/100 ml recorded inhibition of *F.udum* in the range of 32.67 to 34.50 per cent at different pH.

Herbicide Pendimethalin @100 µl/100 ml medium exerted adverse effect on *Trichoderma viride* exhibiting it's 71.52 per cent inhibition. Imazethapyr @ 75µl/100 ml had less detrimental effect compared to Pendimethalin and the growth of *T.viride* was inhibited by

36.44 per cent. Combinations of both the herbicides caused 59.58 per cent inhibition of *T.viride* .

In presence of *T.viride* Imazethapyr @ 75 µl/100 ml was found to inhibit the *F.udum* to the extent of 64.44 per cent, followed by combination of Pendimethalin + Imazethapyr with 60.00 per cent inhibition. Herbicides showed less adverse effect on *T.viride* in presence of pathogen compared to detrimental effect on it when grown alone in herbicide incorporated medium.

Seed treatment with Thiram @ 2 gm/kg + Carbendazim 1g/kg seeds showed late initiation of wilting (60.33 DAS) as compared to all other treatments. Seed treatment with Thiram @ 2 gm/kg + Carbendazim 1g/kg recorded minimum wilt incidence of.1.77, 4.45 and 12.47 per cent at 60, 90 and 120 DAS i.e respectively, compared to 5.39 per cent at 60 DAS, 19.57 at 90 DAS and 29.72 per cent at 120 DAS in control .

Minimum number of colonies of *F. udum* in soil were observed in seed treatment with Thiram @ 2 gm/kg + Carbendazim 1g/kg i.e 5.33×10^4 cfu/g soil at 30 DAS compared to 16.00×10^4 cfu/g soil in control. The trend was similar at 60, 90 and 120 DAS. Significant increase in *T.viride* population was seen due to it's seed treatment and soil application.

CHAPTER I

INTRODUCTION

1.1 Background Information

Pigeonpea *Cajanus cajan* (L.) Mill sp. is an important pulse crop or grain legume crop in semiarid tropical and subtropical areas of the world.It comes under family 'Leguminoceae' and supposed to be originated in India. In India commonly known as 'Tur' or 'Red gram' or 'Arhar' is one of the most an important pulse crop grown extensively for its rich protein content (21%) and forms important constituent of Indian vegetarian diet. It is mainly used as 'dal' (dry, dehulled, split seed used for cooking), green seeds as a vegetable, crushed dry seeds as animal feed and green leaves as fodder. As a source of high value protein it plays an important role in the nutrition.

Pigeonpea is a rich source of proteins, carbohydrates and certain minerals. The protein content in different varieties ranges from 15.5 to 28.8 per cent. Pigeonpea seeds also contain about 57.3 to 58.7 per cent carbohydrates, 1.2 to 8.1 per cent crude fiber and 0.6 to 3.8 per cent lipids. Seed of pigeonpea is a good source of iron and iodine and rich in essential amino acids like lycine, tyrocine, cystine and arginine. This crop can be grown on wide range of soil and under diverse agroecological environment.

In India, pigeonpea occupies 4.09 million hectares of area with an annual production of 3.27 M tonnes and productivity of 799 kg/ha. (Anonymous, 2011 a). Major pigeonpea growing states in India includes Uttar Pradesh, Maharashtra, Madhya Pradesh, Gujarat and Karnataka.

Maharashtra accounts for pigeonpea cultivation over an area of 12.23 Lakh ha with annual production of 8.7 Lakh tonnes and average productivity of 706 kg/ha. In Vidarbha, Pigeonpea cultivation over an area of 6.14 Lakh ha with annual production of 4.2 Lakh tonnes and average productivity of 779 kg/ha (Anonymous b ,2011). Pigeonpea is mostly cultivated as an intercrop with soybean, cotton and mungbean under rainfed conditions.

Though, the area and production of pigeonpea in India is more, the productivity is very low due to several reasons, of which the disease problem is major one. Among the various climatic conditions and environmental factors that are responsible for low yields, diseases of crop plants are one of the most important factors. Due to cultivation of pigeonpea in diversified climatic situations and various geographical areas it is attacked by more than 100 pathogens (Nene et al.1989). These include fungi, bacteria, viruses, nematodes and mycoplasma like organisms.

Major diseases of pigeonpea are wilt caused by *Fusarium udum,* stem blight caused by *Phytophthora drechsleri* f. sp. *cajani*, stem canker caused by *Phoma cajani* and *Colletotrichum capsici*, root rot caused by *Macrophomina phaseolina*, leaf spot caused by *Cercospora indica*, bacterial leaf spot and canker caused by *Xanthomonas aoxonopodis* pv *cajani* and sterility mosaic caused by Virus

1.2 Importance of the Study

Wilt caused by *Fusarium udum* Butler, is the most destructive soil and seed borne disease of pigeonpea (Butler, 1910).There may be 100 per cent yield loss due to wilt, when it occurs at pre-pod stage. About 67 per cent losses at pod maturity stage and 29.50 per cent at pre-harvest stage were reported in a susceptible variety (Kannaiyan and Nene, 1981). The continuous cultivation of crop for 2-3 years in the same field leads to more wilting and loss due to wilt disease (Patil et al.1990). The wilt pathogen survives in soil as soil invader for 8 to 20 years.

The highest mortality occurs at flowering to pod filling stage i.e. from October, November onwards and depends on the duration of the variety. Symptoms may occur 4-6 weeks after sowing. Spraying, drenching and soil application of fungicides can control the diseases. But these are not economical and are polluting agents of the environment. Frequent application of fungicides may lead to the development of tolerance in the target organisms.

Biological control is gaining momentum as an essential component of Integrated disease management. Amongst biological agents, at present *Trichoderma viride* is receiving much attention. *T. viride* have an ability to rapidly colonize the roots of host plants and thus compete with the soil borne pathogens for space and food. Hence biological control of plant diseases using (*T. viride*) may be considered as a well combination with chemical fungicides like Thiram and Carbendazim.

In India, the use of herbicides is increasing year after year and a number of new chemicals are introduced into the market regularly. In India's agrochemical market, herbicides constitute the largest consuming agrochemicals with a share of 45% compared to fungicides (26%) and insecticides (26%). The information on the non target effects of these chemicals on the soil microflora including plant pathogens and their effects on plant disease reactions is meagre. Herbicides are mostly used to control the weeds (non crop plants), but they also have non-target effects on plant pathogens present in the soil (V. Madhuri et al. 2013).

Some herbicides interact with the pathogens and cause more injury. As an interesting side effect, the biological activity of herbicides extends beyond their effect on target organisms and herbicides may influence plant-pathogen interactions through their effect on the pathogen, the plant, or on the surrounding soil organisms including symbiotic interactions Smith et al. (1973).

There are only few reports about additive or even synergistic effects of combined applications of herbicides together with fungicides even though these effects can be expected with regard to the data presented by Heydari and Misaghi (2007).

To be effective, herbicides must have strong biological activity against plants. Indirectly through their effects on plants, herbicides can influence almost any process or interaction of the plant, including its susceptibility to plant diseases. In some cases, herbicides also have direct effects on plant pathogens.Aggravation of disease due to herbicide might have been due to increase in number and virulence of the pathogen as suggested by Fletcher (1960).

The pathogen, *F. udum* is soil-borne and survive in the soil for 8 years. Wilt is most destructive disease of Pigeonpea. There were indications that different races of the fungus existed and could have specificity to different cultivars of Pigeonpea. Herbicides now a days are used for the management of weeds. As Pigeonpea is grown on large area in Vidarbha region of Maharashtra, the use of herbicides is also becoming a more popular in this crop.

Whether the herbicides having any effect on soil borne *Fusarium udum* is not studied. In view of this the present study was undertaken to know the effect of herbicides on wilt of Pigeonpea caused by *Fusarium udum.*

1.3 Objectives of the Study

Use of biological agents and chemical fungicides, pre-emergence herbicides in controlling plant diseases has become more attractive due to added benefit of enhanced plant growth besides disease control. Such enhancement and disease control has been observed with biological agents *Trichoderma viride* and chemical fungicides like Thiram and Carbendazim.Herbicides like Imazethapyr, Pendimethalin are used for the control of weeds in Pigeonpea. Whether these herbicides are having any effect on the growth of *F. udum* at different pH and temperature range is not known . Keeping this in view the present investigation entitled"EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*" was carried out with the following objective :

1. To study of the effect of pre and post emergence herbicides on wilt of pigeonpea *in vivo.*
2. To know the effect of pre and post emergence herbicides on *Fusarium udum* at different p^H and temperature *in vitro.*

1.4 Hypothesis

Although numerous control measures have been suggested to alleviate the problem of wilt and to increase productivity of pigeonpea, their success still remains low due to prohibitive cost of the practices and labour constraints of the small and marginal farmers.

These practices are both resource and knowledge intensive and small farmers often find it difficult to control the disease, especially when the rate of field infestation is high. The method of application of effective fungicide like carbendazim (soil drenching) is not practiced by the farmers, whereas herbicides are being applied to soil where the targeted weed also inhabits. If herbicides proved to have any adverse effect on the wilt causing pathogen and compatible with the fungicides, both the chemicals can be combined to have more effect on the targeted organisms as well as added advantage of application of fungicides through soil which is not adopted by the farmers even though have a very good result and thus the constrain of application method can be overcome to some extent.

1.5 Scope and Limitations

Pigeonpea is one of the important pulse crop of India and occupies an important place in the wealth and economy of India. It is also important sources of protein in cereals based vegetarian diet. Protein contain varies from 15.5-28.8 per cent. Pigeonpea occupies 4.09 million ha area with production of 3.27 million tonnes. (Anonymous, 2011).Saving the losses of such valuable commodity is a major scope for the study.

The fungicides like Carbendazim and Thiram are highly effective against *Fusarium udum* providing effective control. However, use of these fungicides possesses the threat of development of resistant strains of the pathogen. The potential environmental benefits of this approach, leading to a reduction in the use of agricultural chemicals and fit with sustainable management practices.

The pathogen is primarily a soil inhabitant, hence controlling the disease is very difficult as no effective chemicals are available at present, even though soil drenching / seed treatment of Thiram, Carbendazim has been successful in controlling the disease, but to a limited extent and also it is not practically feasible on large areas.

The frequent application of fungicides and herbicides to the soil caused environmental hazards resulting water and soil pollution, in addition killing the non-target beneficial microorganisms in soil. Recently, the biocontrol approaches have been initiated by using antagonistic microorganisms to combat the wilt disease in pigeonpea. Secondly the development of resistant varieties is considered as more practicable.

CHAPTER II

REVIEW OF LITERATURE

2.1 Wilt of pigeonpea

Butler (1906) published a detailed account of studies on *Fusarium* species and reported pigeonpea wilt for the first time in India. Later he carried out the isolation, identification and established the causal organism *Fusarium udum* as a new species.

Butler (1910) reported the causal agent of wilt disease of pigeonpea for the first time in India and described the characters of *Fusarium udum*. Later on in 1910 after thorough investigations, he called the fungus *Fusarium udum* and proved it was pathogenic to pigeonpea. The wilt symptoms were observed after one month of inoculation under pot condition.

Patil and Gokhale (1965) found that continuous cultivation of pigeonpea for 2-3 years in the same field leads to more than 50 per cent losses of plants due to wilt disease. They noted that the disease was more conspicuous in adult stage compared to seedling stage.

Khare et al. (1975) reported 100 per cent wilting at maturity due to symptoms at pre-flowering and podding stage in pigeonpea.

Gaur and Sharma (1982) surveyed pigeonpea crop in Rajasthan and stated that *Fusarium* wilt occurred in patches and rows and it was more severe in fields with sole than a mixed crop.

Patil et al. (1990) reported that wilt disease caused losses ranging from 89 to 100 per cent, at pre pod and early pod stages. The rate of wilt incidence was maximum during flowering and pod formation stage.

Raut et al. (1996) observed that the yield losses depend on the stage of crop at which the plant wilts. The losses due to wilt in susceptible, tolerant and resistant cultivars at different stages, ranged from 12.99 to 99.99 per cent. The losses were more at pod filling stage in all the test cultivars i.e. 43.75 to 82.85 per cent.

Rael Karimi et al. (2012) reported that *Fusarium* wilt (*Fusarium udum* Butler) is an important soil borne disease of pigeonpea [*Cajanus cajan* (L.) Millsp], which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas. The soil borne fungus enters the host vascular system at root tips through wounds leading to progressive chlorosis of leaves, branches, wilting and collapse of the root system. Temperature, soil type, water retentive nature of the soil and nutrient availability has been shown to affect *Fusarium* population.

2.2 Symptoms

Mitra et al. (1977) reported that inoculation with *Fusarium oxysporum* f. sp. *udum* reduced plant height, leaf area, root length and dry weight within 10 to 14 days of inoculation when a plant showed little or no wilting of leaves. The pathogen spread extensively in the vascular system both longitudinally and laterally.

Shit and Gupta (1980) reported that seven isolates of *F. udum* from pigeonpea collected from different regions of India varied in cultural characters such as aerial mycelium, texture and their ability to sporulate. Further, they noted that isolates producing scanty mycelium were more pathogenic with no correlation between intensity of sporulation and pathogenicity and thus, suggested the existence of physiologic races of *F. oxysporum* f. sp. *udum* on the basis of pathogenic behavior.

Bobade (1998) reported loss in turgidity of leaves and yellowing in wilted plants, stem and root splitted exhibiting browning of xylem vessels.

Pardey (2001) record the initiation of wilt symptoms within 8 to 10 days after sowing. Initial symptoms were drooping of upper leaves and loss of turgidity of the plant and within 20 to 21 days complete wilting of plants was recorded.

Kumar et al. (2007) observed typical wilt symptoms on seedlings of the susceptible pigeonpea variety (Bahar) which were inoculated separately with 104 isolates of *Fusarium udum*. The first symptom expression was observed after 23 days of inoculation and the range of wilt incidence at 45 DAI was between 13.3 to 100 per cent.

2.3 Use of Fungicides and *Trichoderma*

Bell et al.(1982) reported that the isolates of the *T. harzianum* collected from different soil samples were not equally effective to inhibit the growth of *F. udum*. Further they found that one isolate (T1) among the 10 isolates of this antagonist was effective. So, there is need to search a very specific isolate(s) of *Trichoderma* sp. for successful control of *Fusarium oxysporum* f.sp. *lycopersici*

Sharma and Jain (1984) tested nine isolates of *F. udum* against three fungicides. They were more sensitive to Bavistin (Carbendazim) and MCB differing in their relative sensitivity. They concluded that those two formulations can thus be used against seed borne *Fusarium* spp.

Bahatnagar (1986) reported *T. viride* equally antagonistic to *F. udum in vitro*

Bahatnagar (1986) reported *T. viride* equally antagonistic to *F. udum in vitro*.

Sivan and Chet (1986) found that *Trichoderma* spp. successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon seeds treated with three isolates of *T. viride* reduced the pre- and post- emergence damping off caused by *R. solani* and *F. oxysporum* f. sp. *sesami* under pot culture and field conditions.

Awadalla *et al.* (1988) reported that pedimethalin ,alachlor and quizalofop-p-ethyl showed 100% inhibition followed by oxyflouren (96.66%), atrazine (89.62%), pedimethalin CS (64.07%), Imazethapyr (61.11%) and 2,4-D showed cent per cent inhibition on mycelial growth of *Fusarium udum*.

Mukhopadhay et al.(1989) investigated biocontrol efficiency of *Trichoderma* for the control of wilt and root rot of chickpea and lentil and found that *Trichoderma viride* and *T. harzianum* inhibited the growth of *Fusarium oxysporum* f. sp. *ciceri* and *lentil*.

Calvet et al. (1990) noted the four strains of *Trichoderma* spp. isolated from two organic composts suppressive to *Fusarium oxysporum f. sp. dianghi*, stimulated mycelial growth from *Glomus mosseae* resting spores in monoxenic cultures on water agar. Since the same strains had shown antagonism against two wilt fungi *in vitro* the possibility exists of using fungal populations antagonistic to plant pathogens to stimulate the VAMF in a growing medium.

Ingole (1995) studied the effectiveness of Thiram + Carbendazim (0.15 + 0.15 per cent) and stated that the maximum mycelial growth of *Fusarium udum* was inhibited with these chemicals.

Sumitha and Gaikwad (1995 a) tested the efficiency of some fungicides against *F. udum* in pigeonpea. They observed that linear growth of pathogen in culture was completely inhibited by Bavistin (Carbendazim) Topsin M-70 (Thiophinate methyl) and Thiram each at 0.1%, Captan 0.15% and Dithane Z-78 (Zineb) at 0.3%.Thiram has given best reduction when used as seed treatment and drench.

Padmodaya and Reddy (1996) screened the isolates of *Trichoderma* sp. for their efficiency in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopercisi in vitro* and found that *Trichoderma viride* was highly inhibitory to *F. oxysporum* f. sp. *lycopercisi* in dual culture, followed by *T. harzianum.*

Cohen *et al.*(1996) studied that pre-treatment of muskmelon seedlings with four chloracetamide herbicides at 0.1 ìg g^{-1} reduced the incidence of *Fusarium* wilt by 22-79 per cent, acetachlor reduced *Fusarium* wilt in muskmelon seedlings when applied as a seed treatment (soaking for 5 hr in solution (50 ìg ml^{-1}) and to a lesser extent when applied as foliar spray.

Biswas and Das (1999) reported that *T. harzianum* was most effective antagonist followed by *T. hamatum, T. longiconis* and *T. koningii* among the five species of *Trichoderma* evaluated under *in vitro* dual culture against *F. udum*. They further noted that pigeonpea seed treatment with *T. harzianum* spores failed to reduce wilt, whereas augmentation of soil with *T. harzianum* in maize meal: sand medium @ 40-60 g/kg soil resulted in significant reduction of wilt incidence up to 89 per cent.

Pandey and Upadhyay (2000) stated that pigeonpea seed treatment with Bavistin (0.2 %) was highly effective in reducing the wilt incidence up to 88 per cent, while the Kavach (0.3 %) was less effective with 41 per cent disease control. Whereas, *T. viride* and *T. harzianum* isolates were best among the biocontrol agents. Integration of biocontrol agents with Carbendazim was not beneficial. However, integration of bioagents with Thiram reduced wilt incidence significantly.

Valva et al. (2001) could manage the *Fusarium oxysporum* f. sp. *ciceri* with soil application of *Trichoderma viride* and *T. harzianum* in the sick soil by recording 21.26 per cent control of the disease.

Jayalakshmi et al.(2003) observed that local isolate of T. harzianum (L1) was most promising and showed maximum inhibitory effect on mycelial growth (88.69 %) of *F. udum* of pigeonpea as well as lowest incidence (20.37 %) of wilt in pots.

Mishra et al. (2004) found that, an isolate of *Trichoderma virence* significantly reduced the *Fusarium oxysporum* f. sp. *gladioli* after incorporation at @ 25 g/kg seeds.

Dhar et al. (2006) studied efficacy of competitive bioagents against *Fusarium udum in vitro* using dual culture technique. Three bioagents *viz., Trichoderma viride, T. harzianum* and *Gliocladium virens* varid in their efficacy in relation to *F. udum* isolates. After 98 hours of inoculation low colony growth was recorded in F_{10}, F_{12}, F_{14} and F_{17} isolates in presence of *T. viride* while least growth of F_{14} (16.0 mm) was obtained with *T. harzianum*. The colony growth of F_{10} was on par (18.3-19.0 mm) with all three bioagents

Nikam et al. (2007) studied *cumulative* effect of three *Trichoderma* species. i.e. *T. viride + T. harzianum + T. hamatum* against *F. oxysporium f. sp. ciceri* and found maximum inhibition (87.77%) of the fungus *in vitro*. It was further observed that all three *Trichoderma* sp. could also exert antagonistic effect individually causing 83.33%, 76.66% and 67.77% inhibition of *F. oxysporium* f. sp. *ciceri* by *T. harzianum, T. viride and T. hamatum*, respectively.

Raju et al. (2008) studied three antagonists *viz., T. viride, T. harzianum* and *Pseudomonas fluorescens* and five fungicides *viz.*, Carbendazim, Captan, Dithane Z-78, Thiophanate methyl and Thiram against *Fusarium oxysporum* f. sp. *udum in vitro. T. viride* was best in inhibiting the growth of the pathogen by 73.6 per cent. Among the fungicides Carbendazim, completely inhibited the growth of pathogen at all concentrations (100, 250, 500 ppm).

Shah et al. (2008) explained antagonistic ability of several rhizosphere fungi and different species of *Trichoderma*. *T. harzianum, T. viride* and *Aspergillus* sp. were observed to be highly efficient in restricting the growth of *Fusarium udum*.

Mehta et al. (2010) conducted pigeonpea wilt management trial. The seed treatment to pigeonpea seed with Thiram (3 g/kg seed) prior to sowing and two drenching of Carbendazim, Benomyl and Hexaconazole, first after 15 days of germination and 2nd at 15 days after 1st drenching, which significantly reduced the per cent wilt incidence and significantly increased the grain yield (kg/ha). Two drenching as above of *T. harzianum* also significantly reduced per cent wilt incidence and increased grain yield (569.5 kg/ha).

Rajput et al. (2010) studied the efficacy of biocontrol agents *viz.*, *Trichoderma harzanium, T. viride, Pseudomonas fluroscens* and *Bacillus subtilis* against chickpea wilt complex pathogens *viz.*, *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia bataticola and Sclerotium rolfsii* .Under dual culture, hyphal growth of pathogens was inhibited at the zone of contact with antagonists *T. harzanium* inhibited the maximum radial growth of *Fusarium oxysporum* f. sp. *ciceri* (65.23%) *R. bataticola* (89.67%) and *S. rolfsii* (86.00%).

Kapoor et al. (2010) studied the antagonistic effect of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *udum,* causing wilt disease of pigeonpea. Among all the bioagents, local isolate of *T. viride* (AI) was most promising showing maximum inhibitory effect on test pathogens mycelial growth (87.77%). All bioagents were further tested as seed dressing agents for the control of wilt disease of pigeonpea. The lowest incidence of wilt (20.48%) was observed in the plots treated with local Isolates of *T. viride*.

Druzhinina et al.(2011) were of the opinion that selection of biocontrol agents as well as understanding the mechanisms involved in the antagonistic effect of *Trichoderma* spp. on plant pathogens are important in designing effective and safe biocontrol strategies. Different isolates of

Trichoderma have different combative ability for pathogen; their indirect effects may also vary. Possible mechanisms of antagonism employed by *Trichoderma* spp. includes nutrient and niche competitions, antibiosis by producing volatile components and non-volatile antibiotics that are inhibitory against a range of soil borne fungi, as well as parasitism. Also synergism between different forms of action modes occurs as the natural condition for the biocontrol of fungal pathogens.

Kamdi *et al.* (2012) studied two antagonists, two fungicides and two botanical extracts against *Fusarium oxysporum* f. sp. *ciceri,*causing chickpea wilt. Field studies revealed that Carbendazim seed treatment (@ 2 g/kg seed) exihibited minimum wilt incidence (26.38%) and maximum yield (13.47 qt/ha) followed by *T. viride* + Carbendazim, *T. viride* + Thiram and *T. viride* alone. Bio-agents *Bacillus subtilis* and aqueous plant extracts of *Azadirachta indica* and *Lantana camera* seed treatments were also significant in reducing wilt incidence and more yield as compared to control

2.4 Herbicides and fungicides against *Fusarium udum*

Bliss et al.(1951) reported that selectivity by a herbicide against an organism such as *T. viride* might have important ecological consequences in view of its known antagonism against a number of plant pathogens.

Fletcher (1960) showed the effects of herbicides on soil micro-organism. The herbicides tested had no effect on the saprophytic growth of the pathogen with the exception of glyphosate, which increased its growth in unsterilized soil. The application of diquat + paraquat and glyphosate to unsterile soil had no effect on the number of actinomycetes. The diquat + paraquat treatment, however, increased populations of fungi while the glyphosate decreased the number of bacteria. The proportion of soil fungi antagonistic to the pathogen was reduced in glyphosate-treated soil.

Kaufmann (1964) reported that application of Simazine, Atrazine, linuron, diuron to soil stimulated fungi known to be antagonistic to *Fusarium* species.

Audus et al. (1966) observed that applications of herbicides at normal field rates did not cause marked changes in the total number of soil microorganisms or in the levels of microbial activity.

Huber et al. (1966) found that decrease in the incidence of various plant diseases due to herbicide application might be due to the effect of the herbicides on the host or pathogen or surrounding microorganisms.

Katan *et al* (1973) reported that herbicides were known to increase or decrease some plant diseases especially those caused by soil borne pathogens.

Griestein et al. (1976) reported the favoured growth of both *F. oxysporum* f. *phaseoli* and *F.solani* f. *phaseoli* using Treflan in bean cultivation.

Kataria and Dodan (1982) observed that herbicides affect the growth of fungal plant pathogens. Results of the study showed that sporangia production was completely inhibited at 50 µl/ a.i.l and 100 µl/a.i.l of pendimethalin and fluchoralin, respectively. Regarding fungistatic action of certain herbicides against soil-borne fungi, fluchloralin and alachlor considerably reduced the growth of *Pythium butleri* and *R. solani.*

Vyas et al.(1986) studied the effect of ten herbicides incorporated into PDA at 50, 100 and 500 µg ml-1 and observed reduced mycelial growth and sclerotial formation of *S. rolfsii, M. phaseolina and F. oxysporum* isolated from the soybean cv JS-72-44 *in vitro.*

Reddy and Vir (1991) reported that combination of herbicides fluchloralin and alachlor with certain fungicides like carbendazim, benomyl and carboxin altered their fugicidal action against *Fusarium oxysporum, Sclerotia rolfsii, Alternaria brassicicola* and *Colletotrichum capsici.* They expressed that the mixtures are at times synergistic and more effective.

Smiley and Wilkins (1992) reported that herbicides can modify host plant structure and defense mechanisms which may lead to greater susceptibility to infection.

Dhiman and Sairnbhi (1993) reported antifungal and antibacterial property of Basalin @ 1.87l/ha and Stomp @ 2.5l/ha towards *Alternaria* blight, downy mildew and black rot of cauliflower under field conditions.

Singh (1996) observed that the efficacy of Ridomil MZ-72 was altered in combination with fluchloralin and pendimethalin (53.27 per cent) followed by Apron 35WS (48.22 per cent) and the least effect was recorded with these herbicides in Captafol. Interaction between herbicides and fungicides was significant.

Pastro and March (1999) observed the effects of acetochlor, imazethapyr, metachlor, pendimethalin, trifluralin and mixture acetochlor and imazethapyr on the production and viability of *S. rolfsii* sclerotia *in vitro.* Trifluralin and pendimethalin were the most efficient compounds because they notably reduced the production of viable *sclerotia.*

Sanogo et al. *(*2000) explained the significant increase in disease severity and pathogen isolation frequency after applying some herbicides stating that the pathogen could adsorb herbicide directly from the spray solution or from plant residues that could be translocated from the roots or foliage.Further they stated that the effect of an herbicide on disease levels is not always the same as its effect on pathogen *in vitro* growth studies, because direct contact between pathogen and herbicide would not be as likely to occur under complex natural environmental condition.

Harikrishnan and Yang (2001) conducted an experiment to determine the effect of glyphosate, imazethapyr and pendimethalin on the mycelial growth, sclerotial production and viability of the isolates of *R. solani.* Pendimethalin significantly reduced the mycelial growth of all the three isolates investigated, whereas the effect of the herbicides imazethapyr and glyphosate were not significant. Sclerotial production was affected differently by the three herbicides.

Sushir and Pandey (2001) reported that Fluchloralin, Oxadiazon and Pendimethalin adversely affect the growth of *Trichoderma* spp. by 42.22, 37.77 and 55.55 per cent even at 125 µl/ml, 250 µl /ml and 250 µl/ml, respectively.

Harikrishnan et al. (2001) observed 100 per cent inhibition of *F. udum* due to seven treatments (pendimethalin + captan, pendimethalin+ tebuconazol, imazethapyr +captan, imazethapyr+ tebuconazol, oxyflourfen + captan, oxyflourfen + tebuconazol and tebuconazol), followed by 99.16 %

due to alachlor + captan, 98.33 % due to oxyflourfen + pyraclostrobin, 97.50 % due to alachlor + mancozeb + tebuconazol and imazethapyr +pyrachlosrobin and 96.66 % due to pendimethalin + pyclostrobin and captan also.

Heydari (2003) stated that herbicides are affecting incidence and severity of plant diseases by interacting with plants, pathogens, or other microorganisms .

Ceballos et al. (2006) showed that MCPA increased the severity of *fusarium* root rot in red clover seedlings in a greenhouse study with the same herbicide rate.

Yadav et al.(2006) Studied the effects of pendimethalin, fluchloralin and metachlor,applied at 1,1.5,or 2 kg/ha at pre-emergence (pendimethalin ,metachlor) or pre-plant incorporation in the soil (fluchloralin) on the germination, growth and nodulation of Chickpea (*Cicer arientinum*) cv. Avrodhi. Application of fluchloralin, pendimethalin, metolachlor each @ 1.0,1.5 and 2.0 kg/ha did not affect the germination (%) in chickpea. Fluchloralin applied at 1.0 kg/ha gave growth promotary effect and significantly increased branched plant, dry weight of plant, nodules/plant and dry weight of the nodules.

Gowdar et al. (2006) conducted experiment to test the possibility of combining *Trichoderma harzianum* a potential bio-control agent with fungicides commonly recommended *viz.*, Captan, Thiram, Carbendazim and Thiophanate methyl for seed treatment against soil borne diseases. Maximum inhibition (100%) was recorded with Carbendazim @ 0.1 and 0.2 per cent concentration at 24 hrs. incubation, followed by 96.88 and 88.44 per cent inhibition at 24 hrs. with Thiophanate methyl @ 0.1 and 0.2 per cent concentration, respectively. The tested concentrations of Captan and Thiram @ 0.1 and 0.2 per cent concentration at 72 hrs. incubation period recorded less than 20 per cent inhibition of *T. harzianum*.

Duke et al. (2007) found that number of plant diseases have increased incidence and severity after applying herbicides which interact in several ways with plants and soil microorganisms that can influence the plant-pathogen relationship.

Rai et al (2007) showed that out of eight tested herbicides oxyflourfen ,alachlor ,quizalofop-p-ethyl and 2,4-D sodium salt were significantly superior in inhibiting the radial growth of *Sclerotium rolfsii* by 100 %, followed by pendimethalin (91.47 %) and atrazine (75.92%) but out of these only 2,4-D showed cent per cent inhibition of mucelial growth of *Fusarium udum*.

Ceballos et al. (2011) tested some herbicides such as MCPA,2,4-DB,bentazon against *Fusarium oxysporum*. The herbicide exhibited the strongest inhibitory effect on pathogen development by the application of field recommended rate.

Madhuri (2013) tested compatibility of herbicides with fungicides against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium udum*. The effect of herbicides along with fungicides was tested by poisoned food technique against these three soil borne pathogens under *in vitro* conditions. Out of eight herbicides tested, oxyflourfen, alachlor, quizalofop-p-ethyl and 2,4-D sodium salt were found highly effective in inhibiting the growth of S. rolfsii, whereas pendimethalin, alachlor and quizalofop-p-ethyl recorded cent per cent growth inhibition of *R. solani*. 2,4 D sodium salt recorded hundred percent inhibition in the radial growth of *F. udum*.

2.5 Effect of temperature and pH

Singh *et al.* (1999) observed that the effects of temperature were evident at both the concentrations (1 and 10 µl/ a.i.l) of Fluchloralin and Pendimethalin. Both the herbicides proved most effective at 20^0C and least inhibitory at higher temperature (30^0C) in case of Fluchloralin. There was a decrease in inhibition of mycelial growth on either side of this maximum inhibitory temperature (20^0C) and it was more pronounced in Pendimethalin at 15^0C as compared to 25 and 30^0C.

Pandav (2002) who found pH 5.5 to 7.0 optimum for the growth and sporulation of *Fusarium*.

Ahamad et al. (2002) revealed that a suitable pH for maximum mycelial mass production was 5.5 followed by 5.0 for both species of *F. oxysporum* f. sp. *psidii* and *F. solani* isolates, while maximum sporulation for *F. oxysporum* f. sp. *psidii* and *F. solani* was obtained at a pH of 6.5, followed by a pH of 6.0. Hence, it is clearly indicated that the tested *Fusarium* spp. favoured acidic pH for its growth and sporulation.

Sharma *et al.* (2005) studied the effect of pH on the growth and sporulation of *F. oxysporum* f. sp. *lini* and reported that tested *Fusarium* spp. could sporulate and grew well at 5.5 pH, radial growth was maximum at temperature of 28°C (72.50 mm for both *F.oxysporum* f. sp.*psidii* and *F.solani*), followed by a temperature of 34°C (66.5 mm for *F. oxysporum* f. sp.*psidii* and *F.solani*).Optimum sporulation was recorded at temperature of 34°C, followed by 40 °C,therefore optimum temperature for the best growth was 28 °C ,which was close to room temperature.However, moderate growth and sporulation was recorded at 34°C because it showed maximum colony growth next to a temperature of 28 °Cand highest in conidia production.

Gupta *et al.* (2010) revealed that the pH level significantly differentiates the mycelia growth. The maximum growth was recorded when the pH was at the level of 5.5 (1208 mg for *F. oxysporum f. sp. psidii*; 1275 mg for *F. solani*), followed by a pH of 5.0 (956 mg for *F. oxysporum f. sp. psidii*; 1083 mg for *F. solani*) and then a pH of 6.0 (952 mg for *F. oxysporum f. sp. psidii*; 958 mm for *F. solani*) .Maximum sporulation of *F. oxysporum f. sp. psidii* isolates was recoded at a pH of 6.5 ($4.4x10^5$ per ml for macro-conidia of *F. oxysporum f. sp. psidii*; $4.8x10^5$ per ml for macro-conidia of *F. solani* and $2.8x10^5$ per ml for micro-conidia of *F. oxysporum f. sp. psidii;* 2.9x 10^5 per ml for micro-conidia of *F. solani*), followed by a pH of 6.0 ($3.8x10^5$ per ml for macro-conidia of both *F. oxysporum f. sp. psidii* and *F. solani;* $2.4x10^5$ per ml for micro-conidia of *F. oxysporum f. sp. psidii;* $2.5x10^5$ per ml for micro-conidia of *F. solani*).

El-Shanshoury *et al.*(2012) who too found 66% decrease in colony diameter and dry weight of the pathogen when the concentration of pendimethalin was increased.Other findings are very much similar to that of 20°C.

Khilare and Ahmed (2012) studied the effect of different culture media, pH and temperature levels on mycelia growth of *Fusarium oxysporum* f.sp. *ciceri*. The fungus grew the best on Czapek dox agar and PDA media. The most suitable pH level for growth of fungus was 6.0 and

6.5 with 24.7 conidia/µl. Growth of *F. oxysporum* was maximum at 30^{0}C (24.7 conidia/µl) after seven days of inoculation, which was reduced drastically below 15^{0}C and above 35^{0}C.

Reddy *et al.* (2013) reported that Glyphosate inhibited *M. phaseolina* growth in a linear dose dependent manner when technical grade Glyphosate acid (GlyCry) was used, however, growth was inhibited in an exponential dose dependent manner when a commercial formulation of Glyphosate-potassium salt (Gly-K salt) was used. The Glyphosate GR50 values (Glyphosate concentration required to cause a 50% reduction) in culture radial growth ranged from 0.25 to 9.94 mm among the *M. Phaseolina* isolates, temperatures, and formulations. The three isolates differed in response to various concentrations across the three temperature regimes.

CHAPTER III

MATERIAL AND METHODS

The present investigation on "EFFECT OF HERBICIDES ON WILT OF PIGEONPEA CAUSED BY *Fusarium udum*" was carried out during *Kharif* 2013 *in vitro,* at the Department of Plant Pathology and *in vivo* on field of Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

3.1 Materials

Following materials were used for experimentation.

3.1.1 Pigeonpea seeds

Seeds of wilt succeptible Pigeonpea variety ICP-2376 was obtained from Pulses Research unit, Dr. PDKV, Akola.

3.1.2 Glassware

The glasswares (Borosil make) used during the course of research work were Petriplates , test tubes, conical flasks of 250 ml, 500 ml and 1000 ml, funnel, beakers, pipette, measuring cylinders, slides, cover slips and glass rods were used

3.1.4 Equipments used

Standard laboratory equipments *viz.* autoclave, BOD incubator, laminar airflow, student microscope, refrigerator, hot air oven, digital weighing balance, Bunsen burner, water distillation unit digital colony counter etc. were used.

3.1.5 Chemicals used

Fungicides like Thiram, Carbendazim, and herbicides like Pendemethalin, Imazethapyr and other chemicals such as streptomycin sulphate and mercuric chloride etc. were used during the course of research work which were obtained from Department of Plant Pathology and Pulses Research unit.

3.1.6 Procurement of bio-agent

The bio-control agent *Trichoderma viride* used in the studies was obtained from Department of Plant Pathology Dr. PDKV, Akola

3.1.7 Other materials used

Blotter paper, non absorbent cotton, muslin cloth, polythene bags, cork borer, inoculating needle, forceps, paper bags, potato, dextrose, Agar-Agar, Mortor and pestle, pipettes etc. were used during research programme.

3.1.8 Disease samples

The wilt diseased samples of pigeonpea were collected from experimental plot of Pulses Research Unit Dr. PDKV, Akola. The samples were examined for the presence of pathogen under research microscope and preserved for further investigation.

3.1.9 Soil samples

Soil samples were collected from rhizosphere of pigeonpea experimental plot of Pulses Research Unit Dr. PDKV, Akola. Samples were air dried and kept in polythene bags and further used for counting colonies of the pathogen.

3.2 Methods

3.2.1 Sterilization of glasswares and other materials

The cleaned, dried glassware were sterilized in hot air oven at 180 ^0C for 1 hour. Culture media and distilled water was sterilized in autoclave at 1.05 kg/cm^2 for 20 minutes.

Preperation of Nash media for isolation of *F. udum*

The media was prepared with following ingredients for isolation of *F.udum* from soil.

Peptone	15 g.
Agar-Agar	20 g.
KH_2PO_4	1 g.
$MgSO_4$	0.5 g.
Streptomycin	0.3 g.
Neomycin	0.3 g.
Aureomycin	0.1 g.
PCNB	1 g.

The above ingredients were mixed in one litre of water, heated to mix the ingredients and filled in flask and sterilized in autoclave at 15 psi for 15 min.

3.2.2.Isolation and Maintenance of Culture

Autoclaved medium was poured at the rate of approximately 20 ml per petriplates of 90 mm diameter. The medium was allowed to solidify. Samples showing characteristic symptoms of wilt were collected and subjected to isolation. The root was cut into small pieces and was washed with running tap water. The bits were disinfected in 0.1 per cent Mercuric Chloride ($HgCl_2$) solution for 1 min. and then washed with three changes in sterilized distill water to remove the traces of mercuric chloride. The bits were dried around the flame and then transferred on solidified PDA. Four bits per plate at equidistance were kept in a plate. The plates were incubated at room temperature (28 ± 2^0C). All the operations were carried out aseptically. The fungal growth was examined under microscope and transferred on slant and purified by hyphal tip method. Culture was transferred on PDA slants and used for further study.

3.2.3 Identification of pathogen

The culture of pathogen obtained by tissue isolation was identified on the basis of morphological characteristics and got confirmed as *Fusarium udum.*

3.2.4 Estimation of *F.udum* population from soil by serial dilution method

Preparation of Potato Dextrose Agar media (PDA)

The medium was prepared by using following ingredients.

Peeled Potato	200g
Dextrose	20g
Agar	20g
Distilled water	1000ml

Peeled potato were sliced into pieces and boiled in 500 ml distilled water till properly cooked. The extract was strained through muslin cloth and measured. In the remaining water, after dissolving dextrose and agar, potato extract was added and the volume was made to one liter. The medium was distributed in flasks and tubes and sterilized in autoclave at 1.04 kg/cm^2 for 15 minutes. The slants were used for maintenance of culture and the medium in flasks for isolation of fungus.

Procedure

Collected air dried soil from field for count the population.The initial dilution was prepared by adding 1 g of air dry soil sample from composite sample into 9 ml distilled sterilized water and dilution blank labeled 10^{-1}. Thus diluting original sample 10 times (1:10).Vigorously shaken the dilution on a magnetic shaker for 2-3 minutes to obtaine uniform distribution and release of micro-organism from adhesing soil particles and allowed the soil particles to settle.From the first dilution transferred 1 ml of suspension to the dilution blank number of 10^{-2} by sterile pipette shaken well for minutes. From 10^{-2} suspension transferred 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette. Repeated the procedure till the original sample had been diluted to 10^{-7}, using every time a fresh sterile pipette. From the appropriate dilution (10^{-6} and 10^{-7}) transfered 1 ml of suspension with respective pipette to sterile petriplates. Three petriplates were used for each dilution. Approximately 20 ml melted Nesh medium was added to each petridishes, containing the diluted sample. Mixed the content of each dish by rotating gently to distribute the cell throughout the medium. Allowed the medium to solidify and incubate these plates in a inverted position for 2-7 days at 28±2^{0}C. The 6th and 7th dilution were plated in petriplates containing selective medium for isolating *Fusarium udum.* Well separated individual whitish colonies were marked and detected.

3.5 Poisoned food technique

3.5.1 *in vitro* evalution of fungicides and herbicides against *F.udum*

Efficacy of fungicides *viz.* Thiram, Carbendazim and herbicides *viz.* Pendimethylene,and Imazethapyr was assayed by poisoned food technique using PDA as basic medium. The treatment details are given below

T_1 - Control

T_2 - Thirum (2g) (0.2%) + carbendazim(1g/kg) (0.1%)

T_3 - Thirum (2g) (0.2%) + carbendazim(1g/kg) (0.1%)+ Pendimethalin @1.0 kg a.i./ha(PE) (100µl/100ml)

T_4 - Thirum (2g) (0.2%) + carbendazim(1g/kg) (0.1%)+ Imazethapyr @ 0.75 kg a.i./ha (75µl/100ml)

T_5 - Thirum (2g) (0.2%) + carbendazim (1g/kg) (0.1%) + Pendimethalin 1.0 kg a.i./ha+ Imazethapyr @ 0.75 kg a.i./ha (75µl/100ml)

T_6 - Pendimethalin @1.0 kg a.i./ha(PE) (100µl/100ml)

T_7 - Imazethapyr @ 0.75 kg a.i./ha (75µl/100ml)

T_8 - Pendimethalin 1.0 kg a.i./ha+ Imazethapyr @ 0.75 kg a.i./ha (75µl/100ml)

Calculated amount of fungicides and herbicides were added individually in the autoclaved medium under aseptic conditions having different pH i.e. 5.5, 6.5, 7.5 and 8.5 which was maintained by pouring 1N NaOH and 1N HCL in the medium. The flasks were shaken thoroughly before medium was poured in three replication of petriplates. A disc of 5 mm pure culture of *F. udum* grown on PDA was inoculated in the centre of solidified medium and petriplates were incubated at different temperature i.e. 20^0C,25^0C,30^0C,35^0C,and 40^0C for a period of 7 days. The average colony diameter was measured in mm at 3^{rd}, 5^{th} and 7^{th} day after inoculation. The per cent growth inhibition was calculated using following formula in Completely Randomized Design

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

Where,

I = Per cent inhibition

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.6 *in vitro* compatibility of *Trichoderma viride* with herbicides

Compatibility tests were conducted under *in vitro* condition to find out safer herbicides against *Trichoderma viride*. For this, two herbicides *viz.*Imazethapyr and Pendimethalin were evaluated by poisoned food technique. The treatment details are given below

T1 . Control

T2 - *T. viride* + Pendimethalin @ 100µl/100ml

T_3– *T. viride* + Imazethapyr @ 75µl/100ml

T_4– *T.viride*+ Pendimethalin@100µl/100ml +Imazethapyr @ 75µl/100ml

Herbicides were added to molten PDA just before pouring. The PDA plates amanded with herbicides were inoculated aseptically with *T.viride* by transfering five mm diameter agar disc from fresh culture. Four replications were maintained for each tratement. Unamanded PDA serve as the control. Inoculated petri plates were incubated at 25 ± 1^0C. The radial growth of *T. viride* was measured in all the treatements after seven days and compared with control. The percent inhibition of *T. viride* over untreated control was calculated using the following formula.

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

Where,

I = Per cent inhibition

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.7 *in vitro* efficacy of *Trichoderma* with herbicides against *F.udum*

Efficacy of bioagent the *T.viride* in presence of herbicides *viz.*,Pendimethylene and Imazethapyr was assayed against *F. udum* by dual culture and food poisoned techniques using PDA as a basic medium. The treatment details are given below

T_1 - Control

T_2 - *Trichoderma viride* alone (without herbicide)

T_3 - *T. viride* +*F.udum* + Pendimethalin @ 100µl/100ml

T_4 - *T. viride* + *F.udum* + Imazethapyr @ 75µl/100ml

T_5 - *T. viride*+ *F.udum* + Pendimethalin @ 100µl/100ml +Imazethapyr @ 75µl/100ml

Potato Dextrose Agar was prepared,sterilized with autoclaved and calculated quantity of herbicide was poured in the media like that of poisobn food technique under aseptic condition. Twenty ml of medium was poured in 90 mm pre-sterilized petri- plates and allowed to solidify. Discs of 5 mm from 7 days old growing culture of *F. udum* were placed on one side of the petri-plate. Similarly, 5 mm discs of the *T. viride* were cut from 7 days old culture and was placed on opposite side of the pathogen. Each treatment was replicated four times. The inoculated plates were incubated at 25+1^{0}C in a BOD incubator. On seventh day the growth of *Fusarium udum* was measured and per cent growth inhibition was calculated using following formula.

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

Where,

I = Per cent inhibition

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.8 Field

Experiment details

The study was conducted by using seeds wilt susceptible variety of pigeonpea ICP-2376, under field (Natural epiphytotics) conditions. The experimental details are given below.

1. Year of experiment : *Kharif*, 2013.
2. Design of experiment : Randomized Block Design (RBD).

3. No. of Replications : Three.

4. Crop : Pigeonpea.

5. Variety : ICP-2376

6. Spacing : 60 cm x 20 cm.

7. Plot size (Gross) : 4.8 m x 4.0 m.

8. Plot size (Net) : 3.6 m x 3.6 m

10. Number of treatments : 11 (as below)

Treatment details

T_1 : Untreated (control)

T_2 : Seed treatment withThirum (2g) + Carbendazim(1g/kg)

T_3 : Application of Pendimethalin 1.0 kg a.i./ha(PE)

T_4 : Application of Imazethapyr @ 0.75 kg a.i./ha

T_5 : Application of Pendimethalin 1.0 kg a.i./ha+ Application of Imazethapyr @ 0.75 kg a.i./ha

T_6 : Seed treatment withThirum (2g)+Carbendazim(1g/kg)+ Application of Pendimethalin 1.0 kg a.i./ha(PE)

T_7 : Seed treatment with Thirum (2g) + Carbendazim (1g/kg) + Application of Imazethapyr @ 0.75 kg a.i./ha

T_8 : Seed treatment with Thirum(2g) + Carbendazim (1g/kg) + Application of Imazethapyr @ 0.75 kg a.i./ha+ Application of Pendimethalin @1.0 kg a.i./ha(PE)

T_9 : Seed treatment with *T.viride* @ 4g/kg+ Application of Pendimethalin @ 1.0 kg a.i./ha(PE)

T_{10} : Seed Treatment with *T.viride* @ 4g/kg + Application of Imazethapyr @ 0.75 kg a.i./ha

T_{11} : Seed Treatment with *T.viride* @ 4g/kg + Application of Imazethapyr @ 0.75 kg a.i./ha+ Application of Pendimethalin 1.0 kg a.i./ha(PE)

Observations Recorded

Percent wilt were recorded at 30, 60, 90 and 120 DAS based on symptoms, the wilted plants were recorded and the percent disease incidence was calculated by using the formula mentioned below.

$$PDI = \frac{\text{No. of dead plants}}{\text{No. of total plants}} \times 100$$

3.9 Precaution to eliminate contamination

All isolation and inoculation work of microbial culture was carried out in laminar air flow. The laminar air flow was sterilized by glowing ultraviolate lamp ½ hr prior to commencement of work. The diseased samples were surface sterilized by using 0.1 per cent mercuric chloride solution.

3.10 Stastitical Analysis

The experimental data was statistically analyzed at Computer Centre, PGI, Dr. P.D.K.V., Akola. Field observation of per cent disease incidence were converted in value of arcsine and square root transformation for statistical analysis in Randomized Block Design. Completely Randomized Design used for analysis of the table value of temperature and pH.

CHAPTER IV

RESULTS AND DISCUSSION

The investigation entitiled "Effect of herbicides on wilt of pigeonpea caused by *Fusarium udum*" was carried out during 2013-14. The results and its interpretation are given in this chapter.

4.1 Symptoms of wilt of pigeonpea

The initiation of symptoms of wilting started from 31 to 34 days after sowing. Initial symptoms were the drooping of upper leaves and loss of turgidity of the plant and within 2-3 days complete wilting was observed. When such infected plants were split opened longitudinally, browning of the vascular tissues was noticed. There was blockage of xylem and phloem vessels consisting spores and mycelial strands which could be observed under microscope.

4.2 Morphological and cultural characters of *Fusarium udum*

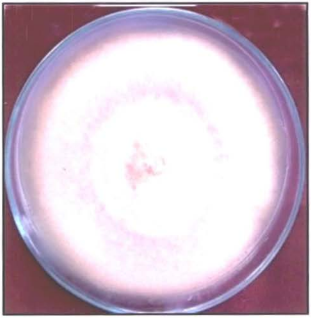
The pathogen was observed under microscope to study it's morphology. Conidia of *Fusarium udum* were borne on slimy sporodochia. Macroconidia were 2-3 septate and occasionally 4-6 septate, thin walled, reiniform falcate, narrowing at both ends. Macroconidia passess prominent hook at the apex and sickle shaped (Plate 1).

The abundance of microconidia were also observed which were 0-1 septate, ovoid and curved shaped and hyaline. The fungus also produced thick walled spherical to oval shaped terminal and intercalary chlamydospores.

4.3 *In vitro* efficacy of fungicides and herbicides against *F.udum* at 20^{0}C and various pH levels

The data presented in Table 1 Plate 2(a) and fig 1 revealed cent per cent inhibition of *Fusarium udum* due to combination of Thiram @ 0.2% and Carbendazim @ 0.1% at 20^{0}C and all the tested pH at 7th DAI. The combination of herbicides and fungicides *viz.*,Thiram+ Carbendazim, Thiram + Carbendazim + Pendimethalin, Thiram+ Carbendazim+

Wilted Pigeonpea plants



Pure culture

Micro and Macro conidia of *Fusarium udum*

Plate 1 Pure culture of *Fusarium udum*

Imazethapyr and Thiram+ Carbendazim +Pendimethalin +Imazethapyr showed 100 per cent inhibition of the pathogen at all four pH levels *i.e.*5.5, 6.5, 7.5 and 8.5, indicating the harmful ion concentration to restrict the growth at 20°C temperature. The pre-emergence herbicide Pendimethalin@ 100µl/100ml medium was also effective in inhibiting the growth of pathogen compared to control and recorded 50.37, 43.71,60.75,and 55.55 per cent inhibition at 5.5, 6.5, 7.5 and 8.5 pH levels, respectively. Imazethapyr @ 75µl/100ml medium also inhibited the growth of pathogen compared to control by 25.93, 40.0, 49.64 and 38.53 per cent at 5.5,6.5,7.5 and 8.5 pH, respectively. Combination of these two herbicides Pendimethalin+ Imazethapyr showed 17.77 to 40.00 % inhibition at different pH levels. It indicated that in acidic to slightly alkaline soil though fungicides reduce the pathogen simultaneously herbicide Pendimethalin and imazethapyr could be able to minimize the wilt pathogen.

The present study indicated that Pendimethalin and imazethapyr can be mixed with these two fungicides for inhibiting the growth of the pathogen. Singh (1999) also observed increased toxicity of two pre-emergence herbicides, Fluchloralin and Pendimethalin with fungicides Apron 35WS and Ridomil MZ, in inhibiting the sporangial development of *Phytophthora drechsleri* f.sp. *cajani*. The fungus had shown growth at all the pH from 5.5 to 8.5 which can be supported by the findings of Nikam (2007) and Pandav (2002) who found pH 5.5 to 7.0 optimum for the growth and sporulation of *Fusarium*.

Table 1. ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 20°C temperature and different pH levels.**

TREATMENTS	Mean colony diameter (mm)	% Inhibition	Mean colony diameter (mm)	% Inhibition	Mean colony diameter (mm))	% Inhibition	Mean colony diameter (mm)	% Inhibition
	pH 5.5		pH 6.5		pH 7.5		pH 8.5	
Thiram@(0.2%)+Carbendazim@ (0.1%)	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Pendimethalin @ 100µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Imazethapyr @ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%) + Pendimethalin @ 100µl/100ml + Imazethapyr @_ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Pendimethalin @100µl/100ml	22.33	50.37	25.33	43.71	17.66	60.75	20.00	55.55
Imazethapyr@75µl/100ml	33.33	25.93	27.00	40.0	22.66	49.64	27.66	38.53
Pendimethalin @100µl/100ml + Imazethapyr @75µl/100ml	37.00	17.77	33.00	26.66	27.00	40.00	35.33	21.45
Control (untreated)	45	-	45	-	45	-	45	-
F Test	Sig	-	Sig	-	Sig	-	Sig	-
S.E.(m) ±	0.57	-	0.9	-	0.9	-	1.16	-
C.D (P=0.01)	2.43	-	3.8	-	3.82	-	4.9	-

Plate 2(a): ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 20°C temperature and different pH ranges.**

T_1 Thiram @ (0.2%)+Carbendazim @ (0.1%)

T_2 Thiram @ (0.2%)+Carbendazim @ (0.1%) +Pendimethalin @ 100µl/100ml

T_3 Thiram @ (0.2%)+Carbendazim @ (0.1%)+Imazethapyr @ 75µl/100ml

T_4 Thiram @ (0.2%)+Carbendazim @ (0.1%) + Pendimethalin @ 100µl/100ml + Imazethapyr @ 75µl/100ml

T_5 Pendimethalin @100µl/100ml

T_6 Imazethapyr@75µl/100ml

T_7 Pendimethalin @100µl/100ml + Imazethapyr @75µl/100ml

T_8 Control

Plate 2(b): ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 25°C temperature and different pH ranges.**

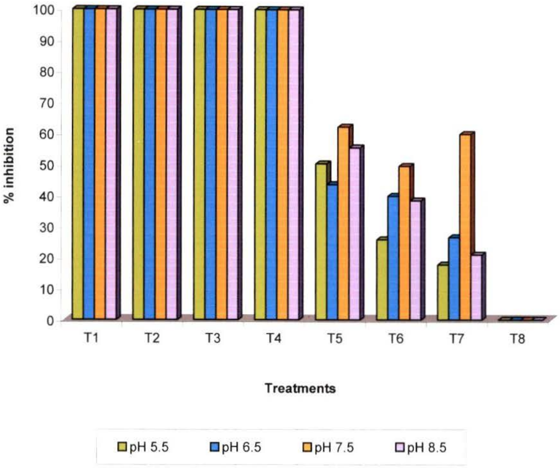


Fig.1.*In vitro* effect of fungicides and herbicides on *Fusarium udum* at 20^{0}C temperature and different pH ranges.

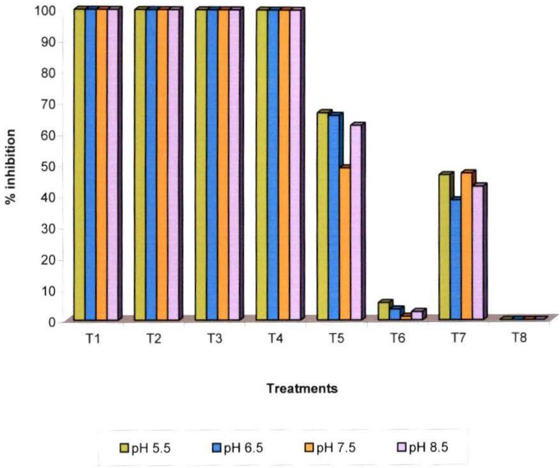


Fig.2.*In vitro* effect of fungicides and herbicides on *Fusarium udum* at 25^{0}C temperature and different pH ranges.

4.3.3 At 30^{0}C and various pH levels

At this temperature also 100 per cent inhibition of *F. udum* was observed due combination of Thiram 0.2 % and Carbendazim 0.1% in all pH at 7th DAI.(Table 3 and Plate 3 (a) and fig.3). The combination of fungicides and herbicide Thiram + Carbendazim + Pendimethalin + Imazethapyr also showed cent per cent inhibition of pathogen at all four pH levels from .5.5 to 8.5, indicating the harmful ion concentration of herbicide to restrict the growth at 30°C temperature. The pre-emergence herbicide Pendimethalin@ 100µl/100ml medium was effective in inhibiting the growth of pathogen to the tune of 62.84, 61.17, 61.18 and 62.35 % at 5.5,6.5,7.5 and 8.5 pH,respectively. At this temperature alone application of Imazethapyr @ 75µl/100ml medium was more effective in inhibiting the growth of pathogen compared to Pendimethalin and recorded 80.45, 81.96, 83.19, and 70.58 % at 5.5,6.5,7.5 and 8.5 pH, respectively. The combination of both the herbicides exhibited inhibition of *F. udum* by 57.75, 58.82, 55.18 and 60.00 %, respectively at 5.5, 6.5, 7.5 and 8.5 pH. Pendimethalin recorded inhibition between ranges of 61.17 to 62.84 % and imazethapyr recorded inhibition between 70.58 to 83.19 % and in combination 55.18 to 60.00% inhibition at different pH ranges. It indicated that at 30^{0}C the herbicide imazethapyr showed more fungitoxicity compared to other temperature range. Singh (1999) was of the opinion that effects of temperature were evident at different concentrations of herbicides. However the tested herbicide in his study proved most effective at 20°C and least inhibitory at higher temperature (30°C). This differences in the result might be due to different herbicides tested in both the studies. On the othe hand herbicides (metribuzin, diuron, eptam, cotoran, cotofor, trifluralin, and pendimethalin) inhibited radial growth, mycelial biomass and spore germination of *Sclerotinia sclerotiorum* and *Verticillium dahliae* as observed by Grinstein et al. (1976); and Awadalla, (1988).

Table 3. *In vitro* effect of fungicides and herbicides on *Fusarium udum* at 30^{0}C temperature and different pH ranges.

TREATMENT	Mean colony diameter (mm)	% Inhibition	Mean colony diameter (mm)	% Inhibition	Mean colony diameter (mm)	% Inhibition	Mean colony diameter (mm)	% Inhibition
	pH 5.5		**pH 6.5**		**pH 7.5**		**pH 8.5**	
Thiram @ (0.2%)+Carbendazim @ (0.1%)	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Pendimethalin @ 100µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Imazethapyr @ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%) + Pendimethalin @ 100µl/100ml + Imazethapyr @ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Pendimethalin @100µl/100ml	31.66	62.84	33.00	61.17	32.33	61.18	32.00	62.35
Imazethapyr@75µl/100ml	16.66	80.45	15.33	81.96	14.00	83.19	25.00	70.58
Pendimethalin @100µl/100ml + Imazethapyr @75µl/100ml	36.00	57.75	35.00	58.82	37.33	55.18	34.00	60.00
Control (untreated)	85.22	-	85.00	-	83.30	-	85.00	-
F Test	Sig	-	Sig.	-	Sig.	-	Sig.	-
S.E.(m) $\pm$	1.01	-	1.30	-	0.95	-	0.86	-
C.D (P=0.01)	4.2	-	5.39	-	3.93	-	3.58	-

Plate 3(a): ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 30°C temperature and different pH ranges.**

T_1 Thiram @ (0.2%)+Carbendazim @ (0.1%)

T_2 Thiram @ (0.2%)+Carbendazim @ (0.1%) +Pendimethalin @ 100μl/100ml

T_3 Thiram @ (0.2%)+Carbendazim @ (0.1%)+Imazethapyr @ 75μl/100ml

T_4 Thiram @ (0.2%)+Carbendazim @ (0.1%) + Pendimethalin @ 100μl/100ml + Imazethapyr @ 75μl/100ml

T_5 Pendimethalin @100μl/100ml

T_6 Imazethapyr@75μl/100ml

T_7 Pendimethalin @100μl/100ml + Imazethapyr @75μl/100ml

T_8 Control

Plate 3(b): ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 35°C temperature and different pH ranges.**

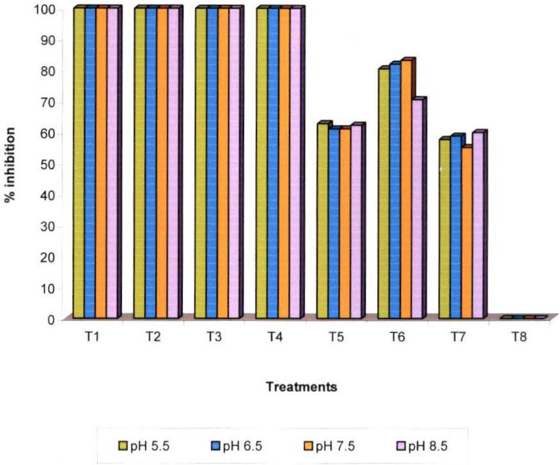


Fig.3.*In vitro* effect of fungicides and herbicides on *Fusarium udum* at 30^{0}C temperature and different pH ranges.

4.3.4 At 35^{0}C and various pH levels

The data presented in Table 4 Plate 3 (b) and fig.4 , revealed same trend of inhibition of *F.udum* due to various treatments at 35^{0}C at 5.5, 6.5, 7.5 and 8.5 pH at 7th DAI. Here also 100 per cent inhibition of *F.udum* was noticed due to Thiram 0.2% and Carbendazim 0.1% . The combination of herbicides and fungicides Thiram+ Carbendazim + Pendimethalin + Imazethapyr also showed cent per cent inhibition of the pathogen at all four pH levels of 5.5 to 8.5 pH. At this temperature also the pre-emergence herbicide Imazethapyr @ 75µl/100ml medium was more effective in inhibiting the growth of the pathogen showing inhibition by 67.99, 68.03, 68.26 and 70.82 %, respectively at 5.5, 6.5, 7.5 and 8.5 pH. Pendimethalin@ 100µl/100ml medium was responsible for inhibiting the growth of the pathogen by 62.88, 68.42, 64.39 and 63.82 % at 5.5, 6.5, 7.5 and 8.5 pH, respectively and in combination of both the herbicides the inhibition was 32.67, 34.50, 33.03 and 33.48 % at 5.5, 6.5, 7.5 and 8.5 pH respectively. Pendimethalin recorded inhibition between range of 62.88 to 68.42 % and imazethapyr recorded inhibition between 68.03 to 70.82% however, inhibition by Imazethapyr was slightly more compared to Pendimethalin at this temperature.

At all the temperature and pH tested, combination of Thiram and Carbendazim proved most effective suppressing the growth of *F. udum* by 100 per cent. Carbendazim and Thiram at various concentration have been found effective against *F.udum* by Somsekhara *et.al.*(2000), Yadav *et.al.*(2007), Raju *et.al.*(2008), Mehta *et.al.*(2010), Andrabi *et.al.*(2011) and Hukma and Pandey (2011). In the present study, the temperature had shown effect on the performance of herbicides whereas pH showed similar trend at all the temperature in inhibiting the growth of *F. udum*.

Table 4. ***In vitro*** **effect of fungicides and herbicides on** ***Fusarium udum*** **at 35°C temperature and different pH levels.**

TREATMENT	Mean colony diameter (mm) pH 5.5	% Inhibition	Mean colony diameter (mm) pH 6.5	% Inhibition	Mean colony diameter (mm) pH 7.5	% Inhibition	Mean colony diameter (mm) pH 8.5	% Inhibition
Thiram @ (0.2%)+Carbendazim @ (0.1%)	0	100.0	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Pendimethalin @ 100µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%)+Imazethapyr @ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Thiram @ (0.2%)+Carbendazim @ (0.1%) + Pendimethalin @ 100µl/100ml + Imazethapyr @ 75µl/100ml	0	100.00	0	100.00	0	100.00	0	100.00
Pendimethalin @100µl/100ml	31.66	62.88	27.00	68.42	30.66	64.39	31.00	63.82
Imazethapyr@75µl/100ml	27.3	67.99	27.33	68.03	27.32	68.26	25.00	70.82
Pendimethalin @100µl/100ml + Imazethapyr @75µl/100ml	57.43	32.67	56.00	34.50	57.66	33.03	57.00	33.48
Control (untreated)	85.30	-	85.50	-	86.10	-	85.70	-
F Test	Sig.	-	Sig.	-	Sig.	-	Sig.	-
S.E.(m) ±	0.42	-	0.4	-	0.67	-	0.62	-
C.D (P=0.01)	1.75	-	1.68	-	2.79	-	2.57	-

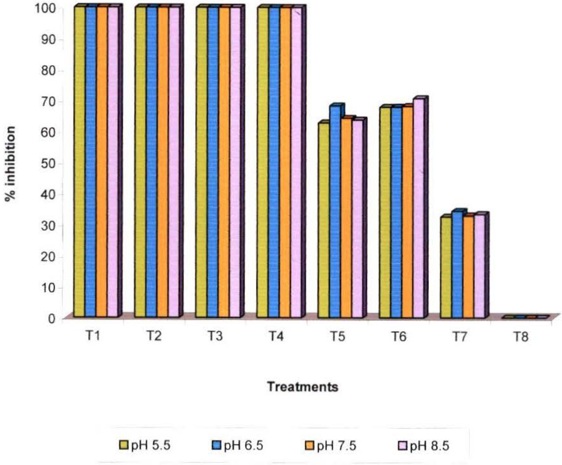
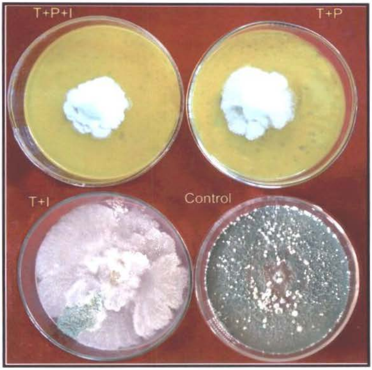


Fig.4.*In vitro* effect of fungicides and herbicides on *Fusarium udum* at 35^{0}C temperature and different pH ranges.



A) *In vitro* compatibility of *Trichoderma viride* with herbicides by poison food technique

B) *In vitro* antagonism of *Trichoderma viride* against *Fusarium udum* with herbicides (Dual culture)

Plate 4: Compatibility of bioagent with herbicides

However, at all the temperatures the growth of the pathogen was inhibited more in acidic pH compared to alkaline, which can be correlated by the findings of many earlier workers that acidic pH favors the multiplication of many species of *Fusarium* including *F. udum,*such as Ahamad et al. (2002), Sharma et al. (2005), Gupta et al. (2010) and Khilare et al. (2012).

The suppression of mycelia growth of *F. udum* by herbicides Imazethaypr and Pendimethalin can be correlated with the findings of Sanogo et al.(2000) who observed that colony growth of the pathogen was most strongly affected on 3% PDA when amended with the herbicides *viz.,*lactofen, glyphosate or imazethapyr and Bhoraniya *et.al.*(2002) who reported that the herbicides fluchloralin and pendimethalin inhibited sclerotial germination of *S.rolfsii* by 94.0 and 94.2%.

4.4 Compatibility of bioagent with herbicides

4.4.1 *In vitro* compatibility of *Trichoderma viride* with herbicides

Compatibility of *T. viride* with herbicides was tested by poisoned food technique , the colony diameter of *Trichoderma* was recorded at 7^{th} DAI and the data is presented in Table 5. PDA amended incorporated with Pendimethalin @100µl/100ml showed 71.52 per cent inhibition and Imazethapyr @75µl/100ml showed 36.44 per cent inhibition of *T. viride* ,whereas it was 59.58 per cent inhibition with combination of both the herbicides. It indicated that the herbicides are not compatible with *T.viride* (Plate 4 (a).

Sushir and Pandey (2001) reported that Fluchloralin, Oxadiazon and Pendimethalin adversely affected the growth of *Trichoderma* spp. Jhonson (2002) also reported 48 per cent growth inhibition of *Trichoderma* spp. with Pendimethalin, These observations confirms the present results.

Ciraj (1996), Wachowska (1999) reported that the growth of saprophytic fungi including *Trichoderma* genus can also limited by herbicides like Gesard 500 SC, Roundup or preparations containing atrazine.

Table 5. *In vitro* Compatibility of *Trichoderma viride* with herbicides

Treatment No.	Treatments	Mycelial growth of *T. viride* (mm)	% inhibition of *T. viride*
T1	Pendimethalin@ 100µl/100 ml	25.62	71.52
T2	Imazethapyr @75µl/100 ml	51.2	43.11
T3	Pendimethalin @100µl/100 ml + Imazethapyr @75µl/100 ml	36.37	59.58
T4	Control (untreated)	90	-
	F test	Sig.	-
	S.E.(m) ±	0.97	-
	C.D (P=0.01)	3.64	-

4.4.2 *In vitro* efficacy of *Trichoderma viride* with herbicides against *F.udum*

In order to assess the ability of antagonist in presence of herbicides against *F.udum* , combination of dual culture and poison food technique was used and the data recorded is presented in Table 6 and Plate 4 (b). Pendimethalin was found to have adverse effect on both the organisms and inhibited the *Fusarium udum* to the extent of 42.22% and that of *T.viride* by 53.10 %. Whereas imazethapyr inhibited *F. udum* to the extent of 64.44% and less adverse effect on *T. viride* with inhibition of 28.40%. In combination of herbicides 60.00% inhibition of the pathogen was recorded as well as the growth of bioagent was also inhibited by 33.95%. These results showed that Pendimethalin and Imazethephyr adversely affected the growth of pathogen as well as *Trichoderma*. However inhibition of the growth of *T.viride* was less when it was tested along with the pathogen, compared to alone compatibility of *Trichoderma* with herbicides. This shows that in presence of both pathogen and antagonist, the herbicides more adversely affected the growth of the pathogen than antagonist.These results are in agreement with the findings of Heydari et al.(1998). They also found that Pendimethalin was significantly more toxic to *Rhizoctonia bataticola* than *Trichoderma* spp, at all the concentrations with 100 % growth inhibition of the pathogen at 500ppm.

Table 6. *In vitro* antagonism of *Trichoderma viride* against *Fusarium udum* with herbicides

TREATMENT	Mycelial growth (mm)		% Inhibition	
	F. udum	*T. viride*	*F. udum*	*T. viride*
Pendimethalin @100µl/100 ml	52.00	42.21	42.22	53.10
Imazethapyr @75µl/100 ml	32.00	64.44	64.44	28.40
Pendimethalin @100µl/100 ml + Imazethapyr @75 µl/100 ml	36.00	59.44	60.00	33.95
T. viride alone (without herbicides)	22.2	83.10	75.33	7.66
Control (untreated)	90	90		
F Test	Sig.	Sig.	-	-
S.E.(m) $\pm$	1.51	1.42	-	-
C.D (P=0.01)	6.551	6.159	-	-

DAI- Days After Inoculation

4.5 Field evaluation of fungicides ,herbicides and *T.viride* against Pigeonpea wilt

A field experiment (Plate 5 and fig.5) was conducted to assess the efficacy of two each fungicides and herbicides and *T.viride* (alone and in combination) against *F.udum* causing Pigeonpea wilt and the results obtained are interpreted below.

4.5.1 Days to initiate wilting

Result (Table 7 and fig.6) revealed that seed treatment with Thirum 2g+ Carbendazim 1g/kg could delay the initiation of wilting,which started after 60.33 days of sowing, compared to 36.33 DAS in untreated control indicating that these combinations could avoid the infection of pathogen for longer time. Second best treatments were T_8,T_7 and T_6, in which the days required to initiate the wilting were 54.66, 54.36 and 54.00, respectively. All these treatments were having thirum and Carbendazim as one of the component alongwith either of the herbicide.Application of the either alone or in combination also resulted in delay of the wilting, compared to untreated control but the delay was less compared to seed treatment with combination of fungicides and they

Fig.5. Plan of Layout of the experimental field

protected the crop from wilting upto 48 days in combination. Although initiation of wilting in pigeonpea at 30 DAS was reported by various workers including Kumar et al. (2007) and Mehta et al. (2010) the delay in wilting due to application of herbicides cannot be correlated with the earlier findings due to lack of literature.

Table 7 Initiation of wilting due to various treatments.

Treatment No.	Treatments	DAS
T_1	Untreated (Control)	36.33
T_2	Seed treatment with Thirum 2g+ Carbendazim 1g/kg	60.33
T_3	Application of Pendimethalin 1.0 kg a.i./ha(PE)	51.33
T_4	Application of Imazethapyr @ 0.75 kg a.i./ha	50.66
T_5	Application of Pendimethalin 1.0 kg a.i./ha+ Application of Imazethapyr @ 0.75 kg a.i./ha	48.00
T_6	Seed treatment withThirum(2g)+Carbendazim(1g/kg) Application of Pendimethalin 1.0 kg a.i./ha(PE)	54.00
T_7	Seed treatment with Thirum(2g)+ Carbendazim (1g) + Application of Imazethapyr @ 0.75 kg a.i./ha	54.36
T_8	Seed treatment with Thirum(2g) + Carbendazim(1g) + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin1.0 kg a.i./ha(PE)	54.66
T_9	Seed treatment with of *Trichoderma* @4g/Kg+ Application of Pendimethalin 1.0 kg a.i./ha(PE)	53.66
T_{10}	Seed Treatment of *Trichoderma* @4 g/kg+Application of Imazethapyr @ 0.75 kg a.i./ha	48.33
T_{11}	Seed Treatment of *Trichoderma* @4 g/kg+Application of Imazethapyr @ 0.75 kg a.i./ha +Application of Pendimethalin @1.0 kg a.i./ha(PE)	50.33

DAS = Days after sowing

4.5.2 Percent wilting at 30, 60, 90 and 120 DAS

Percent wilting at 30, 60, 90 and 120 days after sowing (DAS) in various treatments were recorded and presented in table 8

At 30 DAS, the wilt was not observed even in untreated control and the incidence recorded was 0 per cent in all the treatment as the initiation of wilting in control was 36.33 DAS (Table 7)

At 60 DAS, minimum wilt incidence (0.84%) was recoreded in treatment T_6 (Seed treatment with Thiram @ 2 g/kg Carbendazim @ 1 g/kg

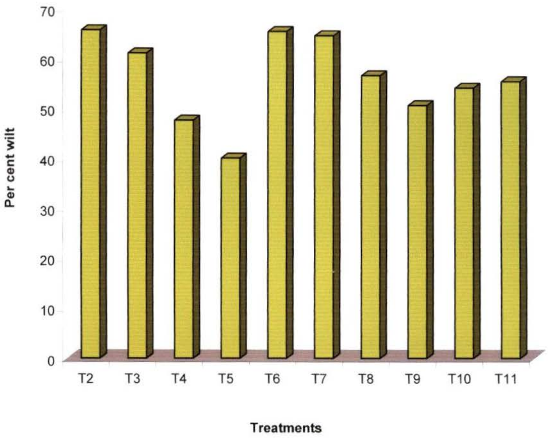


Fig.6. Effect of different treatments on incidence of wilt of pigeonpea

Plate 5. Field view of experimental plot

+ soil application of Pendimethalin 1.0 kg a.i./ha(PE), followed by T_7 (Seed treatment with Thiram @ 2 g/kg+Carbendazim @ 1 g/kg+ Imazethapyr @ 0.75 kg a.i./ha),with 0.92% wilt incidence. The wilt incidence was 1.77% in the treatment T_2 (Seed treatment with Thiram @ 2g /kg + Carbendazim @ 1 g/kg seed) which was more than in treatment T_3 (Application of Pendimethalin 1.0 kg a.i./ha,PE), indicating that herbicide alone could make the plant wilt free initially.

At 90 DAS, treatment T_2 i.e seed treatment with Thiram @2 g/kg + Carbendazim @ 1 g/kg of seed exhibited minimum wilt (4.45%), followed by T_6 and T_7 where the same combination of fungicides were coupled with herbicides i.e. Pendimethalin and Imazethepyr (5.06 % and 5.20% wilting, respectively) .

Similar trend was observwd at 120 DAS, where minimum wilt was due to application of Thiram 2 g/kg +Carbendazim1g/kg of seed (12.47%), followed by T_6 (12.93 %) and T_7 (13.14%).The incidence of wilt was ranged between 18.73 to 22.33%,when the seeds were treated with *T.viride and* combined with herbicides indicating that the antagonistic effect was reduced to some extent as there was some increase in the wilt incidence .

Seed dressing with fungicides along with application of herbicides favoured and enhanced the efficiency of fungicides in reducing the wilt, under field condition.

Cohen et al.(1996) reported the decrease of vascular wilt disease caused by *Fusarium oxysporum* in melon due to acetochlor treatment which was due to direct fungitoxic effect of the herbicide, but the mechanism was not determined. The study showed that a related herbicide, pendimethalin, induces the synthesis of the phytoalexin tomatine in tomato (El-Shanshoury et al.1995). Plants sprayed with imazethapyr and glyphosate, disease severity and isolation frequency from roots were significantly increased compared with the controls and lactofen treated plants as reported by Sanogo et al. (2000). The above findings confirms the present results. However the findings that at 2 WAT, the bensulide, imazethapyr, napropamide, oxadiazon, pendimethalin, simazine, and sulfometuro treatments reduced the number of roots produced Yang (2002) and Bradley (2002) reported that soybean treated with imazethapyr and acifluorfen increased the *Rhizoctonia* disease severity both in greenhouse and field experiments.

Table 8 . Effect of different treatment on wilt of pigeonpea observed at different intervals.

Treatment no.	Treatment detail	Percent wilting				% wilt reduction
		30 DAS	60 DAS	90 DAS	120 DAS	
T1	Untreated (Control)	0	5.39 (2.33)*	19.57 (4.44)*	29.72 (5.45)*	-
T2	Seed treatment with Thiram (2gm) + carbendazim (1gm/kg)	0	1.77 (1.40)	4.45 (2.22)	12.47 (3.49)	65.80
T3	Application of Pendimethalin@ 1.0 kg a.i./ha(PE)	0	1.22 (1.10)	5.52 (2.34)	14.49 (3.81)	61.19
T4	Application of Imazethapyr @ 0.75 kg a.i./ha	0	2.21 (1.50)	7.00 (2.67)	19.38 (4.40)	47.69
T5	Application of Pendimethalin @1.0 kg a.i./ha+ Imazethapyr @ 0.75 kg a.i./ha	0	2.60 (1.59)	7.57 (2.72)	22.39 (4.73)	40.12
T6	Seed treatment withThiram(2gm) + carbendazim(1gm/kg)+ Pendimethalin @1.0 kg a.i./ha(PE)	0	0.84 (0.90)	5.06 (2.21)	12.93 (3.59)	65.58
T7	Seed treatment with Thiram + carbendazim + Application of Imazethapyr @ 0.75 kg a.i./ha	0	0.92 (0.93)	5.20 (2.22)	13.14 (3.62)	64.76
T8	Seed treatment with Thiram + carbendazim + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin@1.0 kg a.i./ha(PE)	0	1.48 (1.23)	5.77 (2.43)	16.40 (4.04)	56.75
T9	Seed treatment with of *Trichoderma*@4g/Kg+ Application of Pendimethalin @1.0 kg a.i./ha(PE)	0	1.50 (1.24)	6.69 (2.60)	18.73 (4.32)	50.76
T10	Seed Treatment of *Trichoderma* @4g/Kg + Application of Imazethapyr @ 0.75 kg a.i./ha	0	1.65 (1.25)	6.50 (2.56)	16.85 (4.10)	54.28
T11	Seed Treatment of *Trichoderma* @4g/Kg + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin @1.0 kg a.i./ha(PE)	0	1.47 (1.22)	7.10 (2.64)	14.60 (3.82)	55.59
F-Test		-	Sig.	Sig.	Sig.	-
SE (m)±		-	0.037	0.06	0.11	-
CD(P=0.05)		-	0.11	0.20	0.33	-

DAS- Days after sowing * Values in the parentheses are square root transformed.

4.5.3 Rhizospheric population of *Fusarium udum* influenced by different treatments

Rhizospheric population of *F. udum* was recorded at 30,60, 90 and 120 DAS. (Table 9). The results were significant at 30 ,60,90 and 120 DAS at 1 per cent level of significance

Population of *F. udum* at 30 DAS was ranged from 5.33 to 16.00×10^4 cfu /g of soil. The drastic reduction in *F. udum* population was observed at 30 DAS in seed treatment with Thiram @ 2g/kg +Carbendazim @ 1 g/kg (5.33×10^4) compared to 16.00×10^4 in untreated control. The population reached to 36.0×10^6 cfu/g soil at 90 DAS in untreated plots and was least in T_7 and T_8 i.e. 6.66×10^4 cfu/g soil in each, while in treatments T_2,and T_6, i.e. combination of fungicides either or along with herbicides the population was slightly more (8.00×10^4 cfu/g soil each). At 120 days, minimum population of 5.00×10^4 cfu/g of soil was observed in T_2 (Seed treatment with Thiram 2 g + Carbendazim 1 g/kg of seed). The present study indicated increased population of *F. udum* from 30 to 120 DAS.The findings correlates the observations of Gade et al. (2007) and Telangre (2010). The results of present study indicating suppression of *F.udum* population due to herbicides are in agreement with the findings of Kaufmann (1964), Reddy and Vir (1991), Harikrishnan et al. (2001), Ceballos et al. (2006), Rai et al. (2007) and Madhuri (2013).

Table 9.Rhizospheric population of *Fusarium udum* in different treatments

Treatment No.	Treatment details	Rhizospheric population of *F. udum* (x 10^4 cfu / g soil)			
		30 DAS	60 DAS	90 DAS	120 DAS
T_1	Untreated (Control)	16.00	21.33	36.00	44.33
T_2	Seed treatment with Thirum 2g +Carbendazim 1g/kg	5.33	10.66	8.00	5.00
T_3	Application of Pendimethalin 1.0 kg a.i./ha(PE)	11.66	14.66	21.00	30.33
T_4	Application of Imazethapyr @ 0.75 kga.i./ha	14.33	19.66	24.00	28
T_5	Application of Pendimethalin 1.0 kg a.i./ha+ Imazethapyr @ 0.75 kg a.i./ha	11.66	15.66	25.00	31.00
T_6	Seed treatment withThirum(2g) + Carbendazim (1g/kg)+ Pendimethalin 1.0 kg a.i./ha(PE)	6.66	10.33	8.00	5.33
T_7	Seed treatment with Thirum (2g) + Carbendazim(1g/kg) + Application of Imazethapyr @ 0.75 kg a.i./ha	7.33	11.33	6.66	7.66
T_8	Seed treatment with Thirum (2g) + Carbendazim(1/kg) + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin1.0 kg a.i./ha(PE)	6.33	12.00	6.66	9.66
T_9	Seed treatment with of *Trichoderma*@4g/Kg+ Application of Pendimethalin 1.0 kg a.i./ha(PE)	13.33	15.33	17.00	30.33
T_{10}	Seed Treatment of *Trichoderma*@4g/Kg + Application of Imazethapyr@ 0.75 kg a.i./ha	12.33	16.3	23.66	31.3
T_{11}	Seed Treatment of *Trichoderma* @@4g/Kg +Application of Imazethapyr @ 0.75 kg a.i./ha+ Application of Pendimethalin 1.0 kg a.i./ha(PE)	15.00	12.66	24.33	33.2
	F-Test	Sig.	Sig.	Sig.	Sig.
	SE(m)±	0.68	1.08	1.30	1.29
	CD (P=0.01)	2.02	3.21	3.84	3.83

4.5.4 Effect of different treatments on rhizospheric population of *Trichoderma viride*

Significant increase in *Trichoderma viride* population was seen due to seed treatment and soil application of the antagonist. The population estimated from 30 to 120 DAS. Treatment T_9, T_{10}, T_{11}, T_{12} i.e. seed treatment with *T.viride* alongwith soil application of the herbicides enhanced *T.viride* population at all days of observations and it was 11.00, 13.00, 9.66 and 9.00x10^4 cfu/g, respectively at 120 DAS compared to 8.50 X 10^4 in untreated control.

Table 10. Rhizospheric population of *Trichoderma viride* in different treatments.

Treatment no.	Treatments	Rhizospheric population of *T.viride* (x 104 cfu / g soil) at DAS			
		30 days	60 days	90 days	120 days
T1	Untreated (Control)	1.333	5.00	5.00	8.5
T2	Seed treatment with Thiram (2gm) + carbendazim (1gm/kg)	1.666	3.666	3.666	4.666
T3	Application of Pendimethalin@ 1.0 kg a.i./ha(PE)	3.00	3.00	3.00	3.00
T4	Application of Imazethapyr @ 0.75 kg a.i./ha	2.00	5.666	5.666	4.666
T5	Application of Pendimethalin @1.0 kg a.i./ha+ Imazethapyr @ 0.75 kg a.i./ha	2.00	3.666	3.666	5.666
T6	Seed treatment withThiram(2gm) + carbendazim(1gm/kg)+ Pendimethalin @1.0 kg a.i./ha(PE)	1.333	2.666	2.666	3.666
T7	Seed treatment with Thiram + carbendazim + Application of Imazethapyr @ 0.75 kg a.i./ha	1.666	4.666	4.666	3.333
T8	Seed treatment with Thiram + carbendazim + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin@1.0 kg a.i./ha(PE)	2.00	5.00	5.00	4.00
T9	Seed treatment with of *Trichoderma*@4g/Kg+ Application of Pendimethalin @1.0 kg a.i./ha(PE)	5.00	8.00	8.00	11.00
T10	Seed Treatment of *Trichoderma* @4g/Kg + Application of Imazethapyr @ 0.75 kg a.i./ha	4.666	7.00	7.00	9.666
T11	Seed Treatment of *Trichoderma* @4g/Kg + Application of Imazethapyr @ 0.75 kg a.i./ha+ Pendimethalin @1.0 kg a.i./ha(PE)	2.666	7.666	7.666	9.00
F-Test		Sig.	Sig.	Sig.	Sig
SE (m)±		0.21	0.41	1.20	0.51
CD (P=0.05)		0.64	1.23	3.55	1.51

DAS- Days after sowing

Audus et al. (1966) observed that application of herbicides at normal field rates did not caused marked changes in the total number of soil microorganisms or in the levels of microbial activity. However Sushir and Pandey (2001) found that some herbicides including Pendimethalin had adverse effect on *Trichoderma*.

CHAPTER V

SUMMARY AND CONCLUSION

Pigeonpea is one of the important pulse crop grown in Maharashtra. The wilt of pigeonpea caused by *Fusarium udum* is important and causes severe losses in yield. In the field, the symptoms of disease were drooping of upper leaves and loss of turgidity of the plant. Within 36 days wilting initiation was observed in control (untreated) plots.

Complete inhibition(100%) of mycelial growth of *Fusarium udum* was recorded in PDA amended Thiram @ 0.2 per cent + Carbendazim @ 0.1 per cent, at 7^{th} DAI, followed by herbicide Pendimethalin @ 100 µl/ 100 ml medium and Imazethapyr @75 µl/ 100 ml of medium with growth inhibition of 50.37 to 55.55 and 33.33 to 38.53 per cent and in combination of herbicides the inhibition of the growth of *F. udum* was to the extent of 17.77 to 21.00 per cent at 7^{th} DAI at 20^0C temperature and different levels of pH (5.5,6.5,7.5 and 8.5)

Similarly effect of the fungicides *viz.* thiram and carbendazim, herbicides *viz.* pendimethalin and imazethapyr alone and in combination against *F.udum* at different pH levels and at temperature of 25^0C were observed. All four pH levels 5.5, 6.5, 7.5, and 8.5 indicated harmful ion concentration to restrict the pathogen growth. Pendimethalin exhibited 67.04,66.28,49.21 and 63.03 per cent inhibition respectively at 5.5, 6.5, 7.5 and 8.5 pH, while Imazethapyr was not encouraging in inhibiting the growth and exhibited 5.64, 3.53, 0.80, and 2.72 per cent inhibition at 5.5, 6.5, 7.5 and 8.5 pH, respectively, but in combination of pendimethalin + imazethapyr inhibition of *F.udum* was recorded between the range of 38.82 to 47.86 per cent at different pH ranges.

Effect of fungicides and herbicides against *F.udum* at 30^0C at different pH showed all the combinations of fungicides along with herbicides having detrimental effect and recorded 100 per cent inhibition of the pathogen at all pH levels 5.5, 6.5, 7.5, 8.5 Pendimathalin exhibited 62.84, 61.67, 61.18 and 62.35 per cent inhibition ,while at this temperature the imzethapyr were less effective and exhibited 80.45, 81.96, 83.19 and 70.58 per cent inhibition at 5.5, 6.5, 7.5 and 8.5 pH, respectively. The

Pendimethalin+ Imazethapyr recorded inhibition of F. udum between the range of 55.18 to 60.00 per cent at various pH.

At 35°C temperature fungicides (Thiram @ 0.2% and Carbendazim @ 0.1%) + herbicides (pendimethalin 100µl/100 ml and imazethapyr 75µl/100 ml) completely inhibited the pathogen. Herbicide Pendimethalin alone inhibited the pathogen by 62.88, 68.42,64.39 and 63.82 per cent at 5.5, 6.5 7.5 and 8.5 pH, respectively whereas Imazethapyr showed 67.99, 68.03,68.26 and 70.82 per cent growth inhibition at the pH levels of 5.5,.5,.5 and 8.5,respectively . The combination of two herbicides were found superior compared to control giving 32.67 to 34.50 per cent inhibition of mycelial growth of F. udum.

Compatibility of T.viride was tested with herbicides viz. pendimethalin and imazethapyr. The colony diameter of T.viride was recorded at 7th DAI, Pendimethalin @ 1.0 kg a.i./ha(PE) i.e 100 µl/100 ml of medium was found highly inhibitory to T.viride resulting 71.52 per cent growth inhibition at 7th DAI. The combination of herbicides exhibited 59.58 per cent inhibition. Imazethapyr @ 0.075 kg a.i./ha i.e. 75 µl/100 ml medium showed 43.11 per cent inhibition, indicating that imazethapyr is less detrimental to T.viride.compared to Pendimethalin.

In vitro antagonism of T.viride against F.udum along with imazethapyr @ 75 µl/100 ml exhibited suppression of F. udum to the extent of 64.44 per cent, followed pendimethalin @ 100 µl/100 ml of media + imazethapyr @ 75µl/100 ml of medium giving 60.00 per cent mycelia inhibition of F.udum.T.viride without herbicide suppressed the growth of pathogen by 75.33 per cent.

Wilting was initiated at 36.33 DAS in control,whereas it was delayed due to various treatments and maximum delay was due to seed treatment of Thiram @ 2 g/kg + Carbendazim @ 1 g/kg, in which wilting was initiated at 60.33 DAS.

Per cent wilt incidence was worked out at 30, 60, 90 and 120 DAS. Minimum wilt incidence was recorded in seed treatment with Thiram @ 2 g/kg + Carbendazim @ 1 g/kg at 90 and 120 DAS i.e.4.45 and12.47 per cent, respectively.

The rhizosphere population of *Fusarium udum* in different treatments varied at 30 to 120 DAS.The minimum population was recorded in seed treatment with Thirum (2g) + Carbendazim (1g) /kg of seed where the population was 5.33 X 10^6 , 10.66 X 10^6 ,8.00 X 10^6 and 5.00 X 10^6 cfu/g of soil at 30, 60 90 and 120 DAS, respectively.

Conclusions :

1. *In vitro* evaluation the test fungicides showed 100 per cent growth inhibition of *F.udum* by Thiram @ 0.2 per cent and Carbendazim@ 0.1 per cent.
2. *Trichoderma viride* showed growth inhibition of *F. udum* by 64.44 % with imazethapyr @ 75µl/100 ml medium, whereas alone it could inhibit the *F. udum* by 75.33 per cent showing suppressive antagonistic effect due to presence of herbicide.
3. Pendimethalin @100 µl/100ml medium and Imazethapyr @ 75µl/100 ml medium exhibited detrimental effect on the bioagent *T.viride*.
4. Seed treatment with Thiram @ 2g/kg + Carbendazim @ 1 g/kg showed delayed initiation of wilting (60 DAS) as compared to all other treatments.
5. Seed treatment with Thiram @ 2 g/kg + Carbendazim @ 1 g/kg + soil application of pendimethalin@1.0 kg a.i./ha(PE) showed minimum wilt incidence at 90 and 120 DAS i.e. 5.06 and 12.93 per cent, respectively.
6. Soil application of herbicides *i.e.* pendimethalin @ 1.0 kg a.i./ha(PE),Imazethapyr@ 0.075 kg a.i./ha alone can reduce wilt to some extent and these herbicides can be incorporated as a component of integrated disease management for wilt of pigeonpea apart from effective contol of the weeds in the field.

CHAPTER VI

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APPENDIX

Media

Potato Dextrose Agar media (PDA)

1. Peeled Potato 200g
2. Dextrose 20g
3. Agar 20g
4. Distilled water 1000ml

Preperation of media (Nash media) for 1 litre water

1. Peptone 15 gm
2. Agar-Agar 20 gm
3. KH_2PO_4 1 gm
4. $MgSO_4$ 0.5 gm
5. Streptomycin 0.3 gm
6. Neomycin 0.3 gm
7. Aureomycin 0.1 gm
8. PCNB 1 GM

Fungicides

1. Carbendazim 50 WP
2. Thiram

Herbicides

1. Pendimethalin
2. Imazethapyr