Two Prominent Entomopathogenic fungi *Beauveria bassiana*(Balsamo) Vuillemin and*Metarhizium anisopliae*(Metchnikoff) Sorokin as Natural enemies of Lepidopteran larvaefromRayalaseema regionofAndhra pradesh

ABSTRACT

Roving survey was conducted for searching the natural occurrence of *Beauveria bassiana*and*Metarhizium anisopliae* during *kharif* season of 2022 and *rabi* season of 2022-23 in Kurnool,Kadapa, Chittoorand Anantapuramudistrictsof Andhra pradesh. Thecrops surveyed wereGroundnut, Maize, Cotton, greengram and blackgram during September to October of *kharif*season and January to February of *rabi* season. Among the crops most of the pest population wasfound in groundnut are*Spodoptera litura*, *Helicoverpaarmigera*and *Aproaremamodicella* andmaize, *Spodoptera frugiperda*which were found at vegetative stage. The fungal infectedcadavers of lepidopteran lavae were observed on the soil below the canopy or adhering to thefoliage of groundnut and maize crop. The larval cadavers were collected in separate aseptic vialsand soil samples were also collected from the surveyed fields. During the survey, it was foundthat the mean number of infected cadavers varied from 2.6 to 3.2 per square meter in Kurnooldistrict during Sep-Oct of 2022. In Anantapuramu district the mean number of cadavers were1.4-2.2 during Jan-Feb of 2023. In Chittoor district the mean number of cadaverswere variedfrom 2.4-3.6, whereas in Kadapa district it is 0.2 per square meter during Jan-Feb of 2023. Thecadaverswereculturedinthelabandbasedonmorphologicalcharactersconfirmedas*Beauveriabassiana*and *Metarhizium anispoliae*.The cadavers of *B.bassiana*were foundto be high inKurnooldistrictfollowedby ChittoordistrictandleastinKadapa districtwhere asthe cadavers of*M.anispoliae*were found to be higher in Chittoor district followed by Anantapuramu district andNilin Kadapadistrict.

Keywords: Survey,Occurrence, Entomopathogenic fungi, *Beauveriabassiana,Metarhiziumanisopliae*

Comment [Omoniyi A1]: Occurrence of Entomopathogenic fungi(Beauvaria bassiana and Metarrhizium anisopliae), in Selected crop farms in Rayakaseema region of Andhra Pradesh

Comment [Omoniyi A2]: The pest population found in among the groundnut crops were

Comment [Omoniyi A3]: crops

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1. INTRODUCTION

The extensive use of synthetic insecticides led to the development of resistance and resurgence ininsect pests in addition to posing problems like pesticide residues and environmental pollution.Eco-friendlypestmanagementtechniqueshavebeengainingmuchattentionsince5to6decades. In the line of biological control of insects, microbial pathogens such as bacteria, fungi,viruses, nematodes, and protozoans are promising agents. Among the various microbial agents,entomopathogenic fungi are considered as most important. Because the fungal pathogens causingthe disease to the insects are practically more significant as they are epizootic in nature. Fungiunlike bacteria or viruses, do not require ingestion for infection, so the sucking insects are alsotargetedeitherbyprimarycontactorbysecondaryuptakeofthepathogenfromsprayedvegetation. Entomopathogenic fungi are natural enemies of diverse terrestrial arthropods and areimportant regulators of host populations in terrestrial ecosystems (Hajek, 1997). They naturallyoccur in insect hosts as infections; they can be collected in the field and grown in the laboratoryfor the documentation of the fungus. *Beauveria bassiana*have been reported to occur naturally inmore than 700 species of insect hosts (Inglis *et al*., 2001). There are more than 700 species offungi from 9 genera that infect insects (Charnley, 1989). Entomopathogenic hyphomycetes havegreatpotentialasbiologicalcontrolagentsagainstinsectsandasonecomponentwithinintegrated pest management systems. They are being developed worldwide for the control ofmanypestsofagriculturalimportance(Ferron,1985).Entomopathogenicfungiarewidelydistributed in a wide range of habitats including aquatic, forest, agricultural and pasture habitats(Samson *et al*., 1998). However, epizootics caused by entomopathogenic fungi in agriculturalhabitats are more numerous, particularly in temperate regions, than those in other habitats. Theoccurrence of entomopathogenic fungi in India has been reported by many workers (Ambethgarand Loganathan 1998). These findings suggest that these fungi act as mortality factors in Indianinsectpopulations.

*Beauveria bassiana*is the asexual form (anamorph) of *Cordyceps bassiana*fungus, which is thesexualreproducingform(teleomorph).ItisanimportantEPFwhichbelongstotheclassDeuteromycetes. It consists of several species like *B. bassiana*, *B. amorpha*, *B. brongniartii*, *B.calendonica*, of which *B. bassiana*is widely recognized as an insect pathogen. It causes whitemuscardinediseaseininsectslikecaterpillars,whiteflies,aphids,thrips,grasshoppers,andbeetles(Sinha*et al*., 2016).

Metarhizium anisopliae is a well-studied soil-inhabiting fungus, having a worldwide distributionrangingfromthe arctictothe tropics (Senthil-Nathan, 2015).Itiscommonly referred as thegreenmuscardinefungus.Thesusceptibleinsecthostsof*Metarhizium*includebeetles[coleopteran order, primarily scarabidae family], root weevils, flies, thrips,gnats etc. (Azizoglu*et al*., 2020). The commercial and indigenous strains of *Metarhizium* fungus were found to beeffectiveagainstwhitegrubs(Bohara*etal*.,2018).Also,thepodborer(*Helicoverpaarmigera*)

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in chickpeas was effectively controlled by using *M. anisopliae* in a study conducted by Rijal andGodfrey(2018).

TheoccurrenceofentomopathogenicfungiinIndiahasbeenreportedbymanyworkers.However, the abundance and biodiversity of these fungi isless known, which can be exploredand documented with the help of authentic and accurate techniques, therefore a survey wascarried out in various places of Rayalaseema zone in Andhra Pradesh to isolate and identify theindigenousfungal isolates.

2. MATERIALS AND METHODSSurveyforcollectionoftestfungi:

A Roving survey was conducted for the collection of *Beauveria bassiana*and *Metarhiziumanisopliae*on insect pests in field crops of four Groundnut growing districts in Rayalaseemaregion of Andhra Pradesh. The survey was conducted Two times i.e during September-October,*kharif* 2022and January-February, *rabi* 2022-2023. The districts selected in Rayalaseema regionof Andhra Pradesh were Kurnool, Kadapa, Chittoor and Anantapuramu. In the selected districts,two Mandals were selected and two villages were selected from each mandal. From Kurnooldistrict,Pathikonda(ChakarallaandPothirallavillage)andDevanakonda(karivemulaandKapatralla village) mandals were selected. From Anantapuramu district, Gooty (Thondavada andubicherla village) and Bukkarayasamudram (Reddipalle and Rekulakunta village) mandals wereselected.FromChittoordistrict,Gangavaram(DandapalleandGangavaramvillage)andpalamaner (Gundlapalle and Jagamarla village) mandals were selected. From Kadapa district,Chintakommadinne (Utukur and Thadiguntla village) and khajipet (Bhumayapalle and MiduturVillage) mandals were selected.In each village, the major field crops availablein the seasonwere observed for *Beauveria bassiana*and *Metarhizium anisopliae* infected cadavers of insectpests. The crops surveyed were groundnut, Maize, Cotton, Blackgram, Sorghum. Among all thecrops observed most of the pest population was observed in Groundnut and maize crops may bedue to the good crop canopy that maintains favourable microclimate to EPF. Pests observed during surveywere Tobacco caterpillar,*Spodoptera litura*, American boll worm,*Helicoverpaarmigera*and Groundnut leaf miner, *Aproaremamodicella*in groundnut crop and Fall army worm,*Spodopterafrugiperda*in maizecrop.

In Chakaralla and Pothiralla village of Kurnool distrsict most of the pest population found was *S.litura*in Chakaralla village and Aproaremamodicella in Pothiralla village. most of the cadaverswere found to be infected with*Beauveria bassiana*and few were infected with*Metarhizium*Sp.in groundnut crop as the most of the farmers follow groundnut cropping system in thesevillages. From Anantapuramu district Thondapadu, Tadiguntla and Rekulakunta villages *S. litura*population was found to be higher. The cadavers were found infected with *Metarhizium* Sp. andfew were found to be infected with *B. bassiana*. In these villages most of

Comment [Omoniyi A13]: collection of cadavers for the isolation of Beauveria bassiana and Metarhizium anisopliae

Comment [Omoniyi A14]: Was it only ground nut grown in those districts?

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the fields were rainfedand groundnut cropping system is followed. In Chittoor district, Dandapalle and Gangavaramvillages,cropsfoundweregroundnutandcabbage.Thepestsobservedwere*H.armigera*and

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*S. litura*ingroundnutcrop.SimilarlyinGundlapallevillageofPalamaner,higher pestpopulation Of*H. armigera*and *S. litura*was found. Most of the cadavers were found to be infected with *M.anisopliae* and few were found to be infected with *B. bassiana*. From Kadapa district in villageutukur village, the groundnut fields were found to be less infested and only *S. litura*was observed. Thecadaversfound wereveryless and infection is seen onlyfrom*B. bassiana.*

The soil type observed was mostly black soil in Kurnool and Kadapa districts where as inAnantapuramu district, the soil type was both red soil and black soil and In case of Chittoordistrict the soil type was sandy loam.The sampling fields were selected randomly in the village.From the fields, the dead cadavers adhered to the leaves, any plant part with external signs ofmycosis were collected with the help of fine brush into sterilized glass/plastic vials and plasticpetriplates.Aftercollectionofentomopathogenicfungalcadaversintothesterilized vialsandthecontainers were wrapped with parafilm and labelled with the details of date of collection, insectcadaver, crop, village, district and zone. The insect cadavers per square meter were counted atfiveplacesrandomlyin thefield.

Isolationof testfungi:

From Entomopathogenicfungal cadavers collected during survey, the fungus was inoculated onto the SMAY medium in petriplates for confirmation of Entomopathogenic fungi. The cultureswere studied morphologically under research microscope and based on the morphological characters theywere confirmed as *B. bassiana*and *M. anisopliae.* After confirmation, pure cultures of isolateswereand maintained in thelaboratory.

3. RESULTSANDDISCUSSION:

Thepestpopulationobservedin Groudnutandmaizecropsare*Spodopteralitura ,Helicoverpaarmigera*and *Aproaremamodicella*in Groundnut crop and *Spodoptera frugiperda*in maize crop. The mummified lepidopteran larvae of EPF were observed in fields of Groundnutand maize which were either adhering to leaves or present on soil. Most of the cadavers werefound adhering to leaves. Some cadavers were with white fungal mat covered on body excepthead portion and some covered with light green spore mass. Mostly later instars of *Spodopteralitura,Helicoverpaarmigera,Spodopterafrugiperda*and*Aproaremamodicella*wereseena scadavers. While collecting the cadavers, white or green spore mass was also observed on leavesorplantparts.

Mostly the cadavers were observed in Groundnut cropmaybe due to canopy coverage andbuildupofmicroclimatewithin the cropfield thatfavourstheEPFpresentin the soil.

Comment [Omoniyi A15]: These are results so transfer to chapter 4

Comment [Omoniyi A16]: This is description of the study area

Comment [Omoniyi A17]: cadaver

Comment [Omoniyi A18]: Petriplates (P should be in capital letter)

Comment [Omoniyi A19]: Result

Comment [Omoniyi A20]: If it was a population, you have to quantify it.

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IncidenceofEntomopathogenicfungiinfectedinsectlarvaeindifferentdistrictsofRayalaseemaregion

KurnoolDistrict:

The pests observed during survey were *Spodoptera litura*, *Helicoverpaarmigera*and *Aproaremamodicella*inthe fieldsof Groundnut.EPFcadaversofallthreecaterpillarswerefoundingroundnut crop in Chakaralla, Pothiralla, karivemula and Kapatralla ofKurnool districtandmeannumberofcadaverspersquaremeterduringSep2022were3.2,2.4,2.2and0.0respectively. In Kapatralla village no insect cadavers were found, it is mostly may be because ofabsence of fungal inoculum in the soil as the weather parameters are not favourable in comparisionwith the other villages. During Sep 2022 the average maximum, minimum temperatures recordedin Kurnool district were 33°C and 22°C. The average morning and evening relative humiditiesrecorded were 37% and 96% respectively. Rainfall of 80 mm was received during the period.During Jan 2023 temperatures recorded were 34°C and 14°C. The average relative humiditiesrecorded were 14% and 89%. Rainfall was not received during the period. Most of the cadaversCollected from this district are found to be infected with *B. Bassiana*and few cadavers werefoundto be infected with*M. anispolaie*.

AnantapuramuDistrict:

DuringsecondfortnightofSeptember2022,mostofthepestpopulationobservedwas*Spodopteralitura.* EPFcadaversof*Spodopteralitura*wererecordedingroundnutcropinTondapadu and Ubicherla villages of Gooty mandal in Anantapuramu district. The mean numberof cadavers found were 2.2 and 1.4 per square meter respectively. During the survey period theaverage maximum, minimum temperatures recorded in Anantapuramu district were 36°C and22°C.Theaveragemorningandeveningrelativehumiditiesrecordedwere37%and98%respectively. Rainfall of 10 mm was received during the period. During Feb 2023 temperaturesrecorded were 32°C and 12°C. The average relative humidities recorded were 23% and 96%.Rainfall was not received during the period.Most of the cadavers collected from this districtwerefoundto beinfectedwith *M.anisopliae*andfew cadavers wereinfected with*B. bassiana*.

ChittoorDistrict:

The pests observed during the survey were *Spodoptera litura*and *Helicoverpaarmigera*inGroundnut and *Spodoptera frugiperda*in maize crop. EPF cadavers of *Spodoptera litura*and*Helicoverpaarmigera*and*Spodopterafrugiperda*wereobservedingroundnutatDandapalle mandal and the mean numbers of cadavers found were 3.6 per square meter and in Gangavaramvillage of Palamanermanndal the mean number of cadavers found were 2.7 per square meterduring the survey conducted in secondfortnight of October,2022.The average maximum,minimum temperatures recorded in Chittoor district were 30°C and 22°C.

Comment [Omoniyi A21]: Incidence is occurrence, rate or frequency. This was not mentioned in the methodology.

Comment [Omoniyi A22]: Represent the observation for each district on a table and do the statistical comparison to make the research more scientific l.

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The average morningand evening relative humidities recorded were 51% and 91% respectively. Rainfall of 118.2 mmwas received during the period. During Feb 2023 temperatures recorded were 32°C and 16°C.The average relative humidities recorded were 45% and 90%. Rainfall was not received duringthe period. Most of the cadavers collected from this district were found to be infected with *M.anisopliae*and few cadavers wereinfected with*B.bassiana.*

KadapaDistrict:

In this District EPF cadavers were found on *Spodoptera litura*and the infected cadavers wereseen on groundnut leaves and mean number of cadavers per square meter were 0.2 in Utukurmandal.Inothersurveyedvillagesthefieldswereobservedwithlesspestpopulation,maybedue to the use of insecticides. The average maximum, minimum temperatures recorded durimgOct 2022 in Kadapa district were 30°C and 22°C. The average morning and evening relativehumidities recorded were 46% and 75% respectively. Rainfall of 56.78 mm was received duringthe period. During Feb 2023 temperatures recorded were 29°C and 14°C. The average relativehumiditiesrecordedwere 40% and89%. Rainfallwasnotreceived during the period.Thecadaversfound wereveryless and infection is seen onlyfrom*B. bassiana.*

The findings of the present study reveal that considerably the higher density of EPF infectedlepidopteran larvae of 3.6 per square meter was found in Dandapalle village of Chittoor districtand 3.2 per square meter in Chakaralla village of Kurnool district might be due to higher rainfallreceived during Sep 2022 with an amount of 80mm in Kurnool district and 118.2mm in Chittoordistrict that creates good relative humidity of 90-95% and the temperatures are also found to befavourable in these districts. In this zone the usage of synthetic pesticides and fertilizers is verylesswhichcreatesopportunityforthesurvivalofsoilfaunaincludingthesoilbornemicroorganismslikeEPF.

The lower incidence of EPF infected lepidopteran larvae of 0.2 per square meter in Kadapadistrict might be due to lower rainfall of 56mm and relatively less humidity of 40-60% which areunfavourable conditions for infection that generally create conditions that are not suitable for soilmicrobes like EPF. Usually under these conditions the soil inoculum will be generally low whichistheanotherreason for lower incidence.

The results of present studies were similar to Nidhi *et al.* (2018) conducted a survey in theKumaun and Garhwal divisions of Uttarakhand for both infected cadavers and soil samples.Isolation of 52 soil samples showed six entomopathogenic fungi. *B. bassiana*infected cadaverswere collected among which mycosis was greatly pronounced in *Trichoplusiaorichalcea*with *N.rileyi*,followedby*B.bassiana*.Similarly,Dhar*etal*.(2019)isolated13fungalisolates,having characteristics similar *to B. bassiana*from infected cadavers and soil samples collected from thefieldsof different crops in Punjab.

Comment [Omoniyi A23]: For better discussion make the deduction, draw inference and then compare with previous researches. You only made a blanket discussion.

Comment [Omoniyi A24]: Can soil sample be isolated or the fungi in it?

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Abarna andVishnupriya (2022)reported that Natural incidence of EPF was highduring the month of December. Twelve EPF isolates were isolated from the mycosed*Tetranychusurticae*cadavers and found to be infected with *Beauveria bassiana*and Cocoka et al. (2023) surveyedthreelocalities in eastern DR Congo and collected infected cadavers of Fall army worm and earwigsand based on the morphologytheyconfirmed it as*B. bassiana*.

The results are also supported by Manjula *et al*. (2019), who found that 100 per cent Groundnutleaf miner population was mycosed during II FN October andI FN September, 2018. Theblotches of leaf miner looked milky white with cadavers protruding from the blotch. The funguswasinoculated and identified as*Metarhizium anisopliae*.

Velavan *et al.* (2021) collected insect cadavers and soil samples from diferent foresttypes, viz., wet evergreen, moist deciduous, dry deciduous and scrub type in South India andisolated27isolatesof*Metarhiziumanisopliae*whichwereconfirmedbasedonmorphologicalandmolecularstudies.

The present results are in line with Ayele *et al.* (2020), who isolated twenty five *Metarhizium*isolates isolated from different soil types, using the great wax moth, *Galleria mellonella* baitingmethod. Likewise, Elham *et al.* (2018) used the insect baiting approach utilising mealwormlarvae, *Tenebrio molitor*, to isolate EPF from soil samples. The only EPF species detected in soilsamplesfromTaman TropikaKenyirandBRISsoil ofMarangwas*Metarhiziumanisopliae*.

Korosi *et al.* (2019) conducted a survey to isolate and identify the entomopathogenic fungi fromsoil. Out of 240 soil samples taken from eight different vineyard sites, 144 samples (60%) werepositive for the presence of entomopathogenic fungi in the genera of *Beauveria* or *Metarhizium*,with20 samples (8%) containingboth.

As per the current study the maximum Mean no. of cadavers/m^2 area was observed in the monthof September and January. This clearly indicates that moderate temperature along with highhumidityis necessaryforthe growth and development of EPF and to cause natural infection to insects.

Namingof isolates:

Comment [Omoniyi A25]: Did you name the isolatexs or you identified them. please clarify.

Theisolateswerenamedbasedontheirareaofcollection.Atotalofsixisolatesof*B.bassiana*(Fig.1)andsixisolates of*M. anisopliae*(Fig.2)werepurecultured, named and maintained for further research.

*Beauveria bassiana*isolate Dpl.Ctr

*Beauveria bassiana*isolate Plmr.Ctr

*Beauveria bassiana*isolate Pkd.Knl

*Beauveria bassiana*isolate Dvk.Knl

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*Beauveria bassiana*isolate Gty.Atp

*Beauveria bassiana*isolate Utkr.Kdp

Metarhizium anisopliae isolate Dpl.Ctr

Metarhizium anisopliae isolate

plmr.Ctr*Metarhiziumanisopliae* isolate Gty.Atp

Metarhizium anisopliae isolate Rkl.Atp

Metarhizium anisopliae isolate Pkd.Knl

*Metarhiziumanisopliae*isolateDvk.Knl

Table.1 Crops surveyed and the incidence of *Beauveria bassiana* and *Metarhizium anisoplaie* in different groundnut growing regions in Rayalaseema regions of Andhra Pradesh

Comment [Omoniyi A26]: Not mentioned in the results or discussion.

S.no	District	Village	Location	Crop	Cadavers noticed	Meanno.of cadavers/sq.m
1.	Kurnool	Chakaralla	15.3889°N, 77.5562° E	Groundnut	*Spodoptera litura, Aproarema modicella.*	3.2
		Pothiralla	15.3964°N, 77.5008° E	Groundnut	*Spodoptera litura*	2.4
		Karivemula	15.5946°N, 77.6004° E	Groundnut, Cotton	*Helicoverpa armigera*	2.2
		Kapatralla	15.5936°N, 77.6316° E	Groundnut	Nil	
2.	Anantapuramu	Thondavada	13.5935°N, 79.3423° E	Groundnut	*Spodoptera litura*	2.2
		Ubicherla	15.1851°N, 77.6894° E	Groundnut	*Spodoptera litura*	1.4
		Reddipalle	14.0927°N,	Groundnut	Nil	

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			79.2278° E			
		Rekulakunta	14.6938°N, 77.6695° E	Groundnut	*Spodoptera litura*	1.0
3.	Chittoor	Dandapalle	13.2392°N, 78.6966° E	Groundnut	*Aproarema modicella*	3.6
		Gangavaram	16.7339°N, 82.0475° E	Groundnut, cabbage	*Helicoverpa armigera*	2.4
		Gundlapalle	13.4477°N, 79.0363° E	Groundnut	*Spodoptera litura*	2.7
		Jagamarla	13.2215°N, 78.8248° E	Groundnut	Nil	
4.	Kadapa	Utukur	14.4538°N, 78.8123° E	Groundnut, Blackgram	*Spodoptera litura*	0.2
		Thadigotla	14.4950°N, 78.7639° E	Groundnut	Nil	
		Bhumayapal Le	14.6921°N, 78.7306° E	Groundnut, Maize	Nil	
		Miduturu	15.7709°N, 78.2889° E	Groundnut	Nil	

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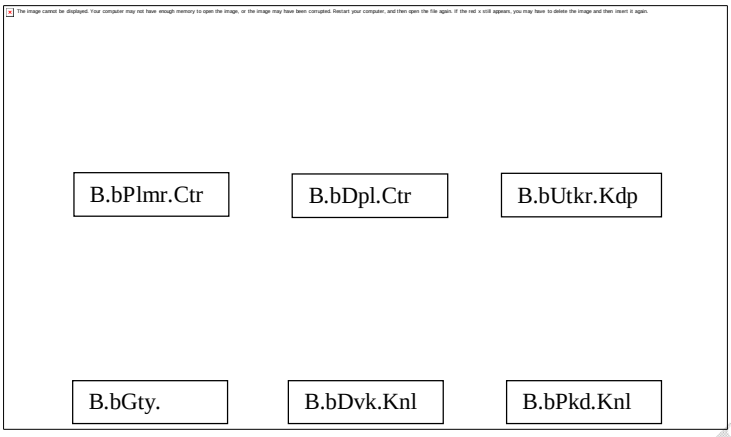

Fig.1 Isolates of *Beauveria bassiana*

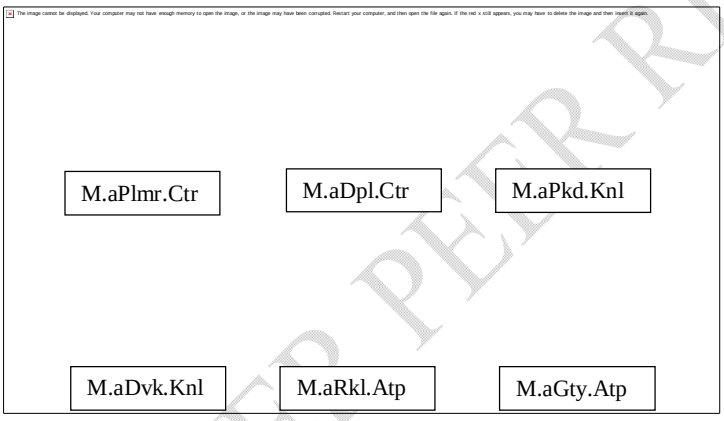

Fig.2 Isolates of *Metarhizium anisopliae*

Comment [Omoniyi A27]: Not referred to in the result or discussion

Fig.3 Graphical representation of incidence of Beauveria bassiana and Metarhizium anisopliae in Rayalaseema zone of Andhra pradesh

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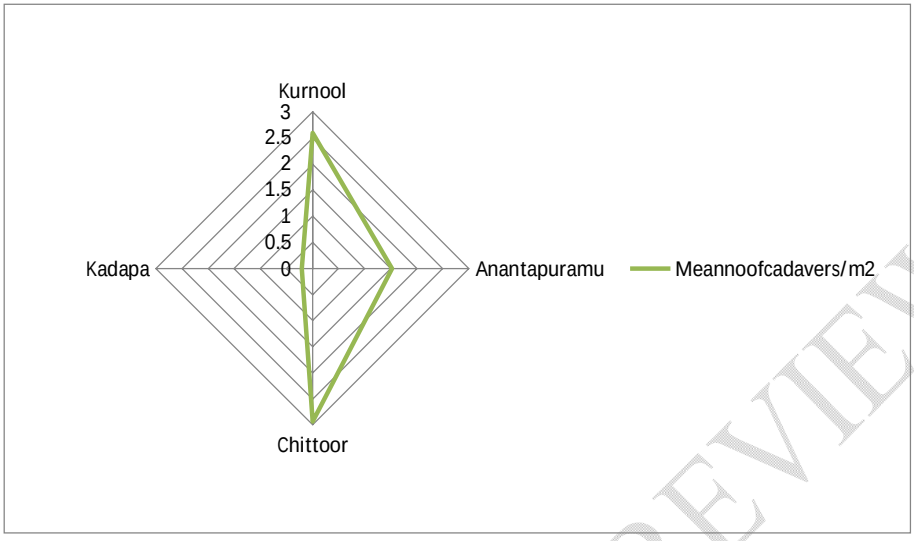

1. CONCLUSIONS

Comment [Omoniyi A28]: State the Conclusion based on the aim or objective of the research not just as you feel.

The findings of the present study reveal that considerably the higher density of Entomopathogenic fungal infectedlepidopteran larvae of 3.6 per square meter was found in Chittoor district and 3.2 per squaremeter inKurnool district and the lower incidence of EPF infected lepidopteran larvae of 0.2 persquaremeterwasfoundin Kadapadistrict. In groundnut and maize crops the major lepidopteran larvae can be suppressed by utilizing these fungi through sole application or by incorporating as a component in IPM strategies.

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Comment [Omoniyi A29]: Use APA format. Please google APA reference format.

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