Effect of various biopesticides and biocides on the leaf pest, *Udaspes folus* of turmeric plants

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ABSTRACT

Entomopathogenic fungi have great potential in pest management due to their mode of action, specificity and cost efficiency. The present study was done to evaluate the effect of various fungal agents like *Trichoderma viride, Beauvaria bassiana* (Balsamo) *Metrarhizium anisophilae* (Metsch), *Hirsutella citriformis*(Speare), *Nomuraea rileyi* (Farlow) Samson and neem based pesticides (Azadiractin) on the leaf roller pest, *Udaspes folus* which affects the growth of turmeric plants to a great extent. The results were compared with the standard chemical pesticide action. The effect of various pesticide applications on turmeric plant and rhizome was also evaluated as the rhizome product is directly consumed by all. The various biochemical parameters in turmeric leaves were studied in both infected and healthy leaves. Among the tested entomopathogenic fungi, *Hirsutella citriformis* was found to significantly control *U. folus* followed by *Metrarhizium anisophilae*.

Key words: Biochemical, biopesticide, entomopathogenic fungi, leaf roller, rhizome

INTRODUCTION

India is a leading producer and exporter of turmeric (*Curcuma longa*. L) in the world. A number of cultivars are available in the country and are known mostly by the name of locality where they are cultivated. Some of the popular cultivars are Duggirala, PTS, Sugandham, Amalapuram, Erode local, Alleppey, Moovattupuzha and Lakadong. The important varieties are Suvarna, Suguna, Sudarsana, IISR Prabha, IISR Prathibha, Co-1, BSR-1, Krishna, Sugandham, Roma, Suroma, Ranga, Rasmi, Rajendra Sonia, IISR Alleppey, Supreme, IISR Kedaram (Kandikannan *et al*., 2008). Turmeric has anti-inflammatory (Punithavathy *et al*., 2000); analgesic, antibacterial, anti-tumor, anti-allergic, anti-oxidant (Menon and Sudhir, 2007) antiseptic, antispasmodic, astringent, digestive, diuretic and stimulant properties. The important diseases caused in the turmeric are anthracnose, leaf blotch, soft rot etc. The anthracose disease is caused by *Colletotrichum capsici*. The pests which attacks are shoot borer, skipper, scale insect, thrip, lacewing bug and leaf beetles. Among the many pests which attack the turmeric, *Thrips* and *Udaspus folus* affects the plant by reducing the surface area of the leaves.
The pest infestation is more common during the post monsoon period especially in drier regions of the country. The turmeric skipper is *Udaspes folus*, a specific and serious pest of turmeric and arrow root. Besides, some of the insecticides reported effective earlier such as carbofuron and quinalophs are now being reported to cause leaf folder resurgence (Nadarajan and Skaria, 1988; Devanesan *et al*., 1995). Biological control, including the use of entomopathogenic fungi, offers a sound alternative management strategy against several pests. Among the entamopathogenic fungi, *Metarhizium anisopliae* is one of the most commonly isolated insect pathogenic fungi with over 200 insect-host species and cosmopolitan distribution (Roberts and Leger, 2004). *Nomuraea rileyi* is an important fungus that attacks larvae of rice insects. It attacks the larvae of stems borers, leaf folders, armyworms, and caseworms. *Beauveria bassiana* is a fungus that grows naturally in soils throughout the world and is being used as a biological insecticide to control a number of pests such as termites, thrips, whiteflies, aphids and different beetles. Along with these fungi, *Hirsutella citriformis* was also used against *U. folus.*

MATERIALS AND METHODS

Collection and rearing of the pest

Among the five larval stages, the second and third larval stages of the *Udaspus folus* were used for the present study and were collected from turmeric fields adjoining the K. S. Rangasamy College of Technology, Tiruchengode, Tamil Nadu, India and maintained in plastic containers. To avoid mortality due to unhygienic conditions the rearing containers were cleaned and fresh turmeric leaves were provided daily during the study period.

Preparation of cultures

For *B. bassiana, T. viridae, H. citriformis and M. anisophilae* Potato Dextrose Agar medium was used and for *N. rileyi*, Saborauds malt agar yeast (SMAY) medium was used. A loop full of fungal culture was inoculated to 200 mL of broth in a conical flask and maintained in a shaking incubator at 28ºC at 120 rpm for ten days. Spore suspension of each pathogenic fungus was prepared by using 80 to 100 mL of sterile distilled water containing 0.05% Tween- 80 solution. The conidial count of cultures were then determined by using a Neubaur hemocytometer and adjusted to 1 x 10^7/mL and stored in 4º C until use. The neem extract was prepared by soaking 200 gm of crushed neem seeds in water for 12 hrs and filtering it. The filtrate was diluted in water and used for the assays.

Larval *in vitro* Assays

The 2nd and 3rd instar larvae were grown in plastic containers covered by a muslin cloth for aeration. Each container consists of ten larvae and three replicates were maintained. 10 mL of spore suspension of the fungi were taken and each larva was dipped for ten seconds. The control larvae were dipped in 0.02% Tween 80 alone. The containers with larvae were maintained at 26 ± 1ºC; relative humidity 70 ± 10% and photoperiod of 16: 8 L: D. Larval mortality was recorded at 24 hrs interval for five days after treatment and the data was analyzed statistically. The cadavers were used for re isolating the pathogen in pure culture for confirming the pathogenicity of fungi. The fungi which showed promising results were tried in field for their efficacy.

Field studies

Field trials were conducted at the turmeric farm in K.S.Rangasamy College of Technology and a turmeric farm in Karungalpalayam, Erode where PTS turmeric was grown in natural conditions during rainy season of 2011 between October-November. The experimental plots were laid in randomized complete block design with eight treatments with a plot size of 10 square meters with thirty plants in each plot and three replicates. The treatments were applied as foliar sprays and comprised as follows: T1-*M. anisophiliae;* T2-*B. bassiana*; T3- *T. viride;* T4-*H. citriformis*; T5- *N. rileyi;* T6–Neem extract; T7- Imidachloprid and T8- control. The spraying of bioformulations was done in the early hrs of the day using a snapsnack sprayer with a spray volume of 300 L ha^{-1}. Five plants were selected randomly from each plot and observed for occurrence of pest and its mortality at frequent intervals. The data was recorded and mortality rate was calculated.

Analysis of biochemical status

Ten grams of fresh and *U. folus* infected leaves were collected from each treatment plot and ground separately with 80% aqueous acetone using a pre chilled mortar and pestle. The aqueous layer was collected in a separate tube and the process was continued till the residue became pale white. The acetone layer were made up to known volume and used for chlorophyll and carotenoids estimation using a UV VIS Spectrophotometer (Hitachi, Japan). The estimation of chlorophyll a, b and total chlorophyll were estimated (Harborne, 1973). Freshly plucked healthy and infected leaves from each plot was ground with aqueous warm 80% ethanol and allowed to boil on a water bath for ten minutes at 50ºC. It was then filtered through two folds of muslin cloth and the resultant solution was used for estimating biochemical parameters. The total carbohydrate and protein was determined by Anthrone and Lowry *et al* methods respectively. Total sugars (Dubois *et al.,* 1956), lipids, amino acids (Moore and Stein, 1948), polyphenols (Bray and Thorpe, 1954) and catechin (Swain and Hillis,

1959) contents were also estimated. The difference in major nutrient NPK and micronutrients like Ca, Zn, Mn and Mg were also studied in both healthy and infected leaves of both turmeric varieties following A.O.A.C methods.

Morphological and yield parameters

The height of the plants, number of leaves affected and disease incidence were calculated in six plants which were selected randomly from each treatment plot. The rhizome yield and its curcumin content (Mahadevan and Sridhar, 1982) after harvest was also studied.

Statistical analysis

The experimental design for all trials was completely randomized. The data were analysed using the analysis of variance (ANOVA) and the mean values were compared by using the Duncan's multiple range tests ($P \leq 0.05$) using statistical package for the social sciences (SPSS) package 11.5 version.

RESULTS

A field trial was conducted to test the bio efficacy of various entomopathogenic fungi against second and third instar larvae of *U. folus* infecting PTS variety turmeric plants which a hybrid cultivar known for its high yield and disease resistance. The *in vitro* studies (Figure 1) showed a significant increase in mortality rate as number of day's caused a death rate of 6.33 and 7.33 in the pest. It was noticed that *M. anisophilae* was efficient next to *H. citriformis* causing mortality at the rate of 2.33 and 4.33 in *in vitro* and 4.67 and 4.7 in field conditions. On the other hand, *T. viride* exhibited low activity against *U. folus* in both lab and field studies with a mortality of only 1.33 at the maximum. The data observed in field conditions (Table 1) show a remarkable death of the pest in treatment 6 with neem extract when compared to other treatments where the mortality was 2.33 in third instar larvae. An increase in disease incidence was noticed in the treatment 6 and in control plots and was low in treatment 4. The effect of fungal treatments on the biochemical parameters of the turmeric leaves vary significantly (Figure 2). The total sugars, total proteins, amino acids, nitrogen, magnesium and manganese levels were found to be higher in treatment 5 with *H. citriformis* whereas the polyphenol and zinc content were found to be less in comparison with other treatments. The amount of catechin was found to be less in all the biocontrol treated plots when compared to the control and chemical pesticide applied plot.

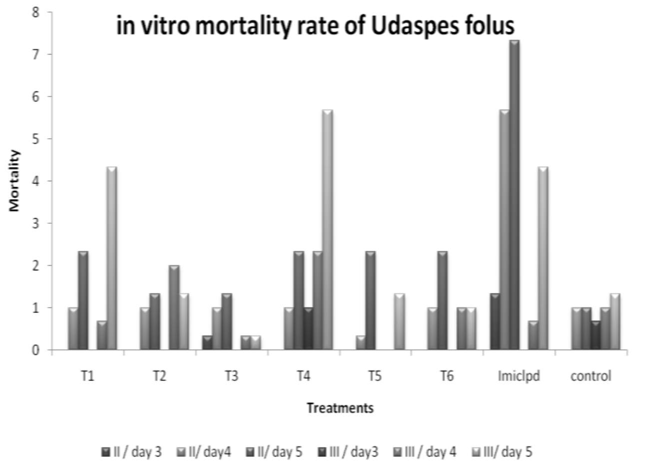

Figure 1. *In vitro* mortality rate of *U. folus* against fungal treatments

Table 1. The mortality of *U. folus* with different fungal formulations in PTS turmeric field

Treatment	II instar larva			III instar larva		
	Day3	Day4	Day5	Day3	Day4	Day5
T1	0.67 ± 0.58^{d}	3.33 ± 0.58^{c}	4.67 ± 0.58^{c}	1.67 ± 0.58^{b}	2 ± 1^{d}	4.7 ± 0.58^{c}
T2	0.67 ± 0.58^{d}	2.33 ± 0.58^{d}	4.33 ± 0.58^{c}	1 ± 1^{d}	3.33 ± 0.58^{f}	4.33 ± 1.15^{d}
T3	0.33 ± 0.58^{e}	1.33 ± 0.58^{f}	2.67 ± 0.58^{d}	0.67 ± 0.58^{de}	2.33 ± 0.58^{c}	2.67 ± 0.58^{e}
T4	1.67 ± 0.58^{b}	4 ± 0^{b}	6.33 ± 0.58^{b}	1.33 ± 0.58^{c}	3.33 ± 0.58^{b}	7.67 ± 0.58^{b}
T5	0.33 ± 0.58^{e}	1.67 ± 0.58^{e}	2.67 ± 0.58^{d}	0.33 ± 0.58^{f}	2 ± 0^{d}	2.33 ± 0.58^{e}
T6	1 ± 0^{c}	1.67 ± 0.58^{e}	2.67 ± 0.58^{d}	1.33 ± 0.58^{c}	2 ± 0^{d}	4.33±0.58d
T7	2.67 ± 0.58^{a}	6 ± 1^{a}	8.67 ± 0.58^{a}	3 ± 0^{a}	6.67 ± 0.58^{a}	9.67 ± 0.58^{a}
T8	0 ± 0^{f}	1 ± 1^{f}	2.33 ± 0.58^{e}	0.33 ± 0.58^{f}	0 ± 0^{e}	1 ± 1^{f}
CD(p=0.05)	0.8	1.12	1.02	0.97	1	0.89

Each value is the average of three observations ± Standard Deviation

Table 2. The effect of fungal applications on various growth and yield characteristics in turmeric plants.

Treatment	Plant height (cm)	No. of Leaves affected	Disease incidence (%)	Fresh rhizome yield (gms)	Curcumin mg /100 g
T1	131±1	2.67±0.58	33.3	428.86±3.25	8±0.1
T2	127.33±0.76	3.67±0.58	37.5	340.84±4.37	8.3±0.1
T3	141.67±1.5	3.00±1	37	426.29±6.23	8.5±0.1
T4	128±2	3.67±0.58	22.2	420.71±1.43	9.1±0.1
T5	130.31.13±	3.33±0.58	36.3	408.05±3.77	8.6±0.1
T6	124.67±2.1	3.67±1.15	50	375.99±12.04	9.0±0.2
T7	135.33±1.1	2.33±0.58	38.4	370.75±11.62	8.7±0.1
T8	137.33±2.1	6.33±0.58	41.6	339.47±9.82	7.8±0.1
CD	4.03	1.29	-	18.90	0.13

Each value is the average of three observations ± Standard Deviation

The amount of chlorophyll a and b, total chlorophyll as well as carotenoids (Figure.3) was found to be the maximum in the treatment 6 in which neem extract was sprayed. Much significant variation was not found in other treatments. The weight of the rhizomes after harvest ranged between 339.47 and 428.86 grams (Table 2). The maximum yield was seen in plots treated with *M. anisopliae* and *H. citriformis* and the minimal yield in the control plots.

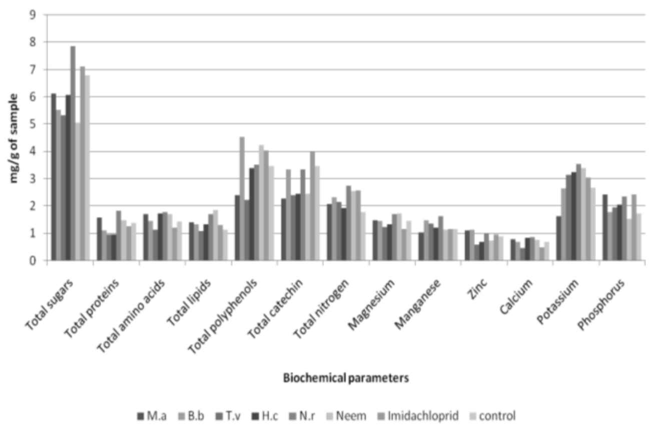

Figure 2. Biochemical parameters of turmeric infected leaves in fungal treatments

DISCUSSION

Biopesticides which are ecofriendly and safe for export crops are of great need. Suman Gupta and Dikshit (2010) indicated that biopesticides can be used as an ecofriendly approach for controlling pests. Among the treatments, maximum reduction of the pest was noticed with treatments where biological control agents were applied as foliar spray. The results of the present study showed that the various fungal cultures inhibited the pest growth in varying degrees. Among the entomopathogenic fungi tested, *H. citriformis* showed maximum efficiency against the leaf roller, *U. folus* and showed promise to be used as a biocontrol agent against this lepidopteran pest. This observation is on par with that of Jason M Meyer *et al*. (2007). Mukhopadhyay *et al*. (2010) studied the control of lepidopteran pests using bacteria and virus. It was evident that even though *Nomuraea rileyi* has been proved as a potential entomopathogenic fungi against Tobacco caterpillar, *Spodoptera litura*, a lepidopteran pest by Shanthakumar *et al*. (2010) its activity was minimal against *U. folus* in turmeric.

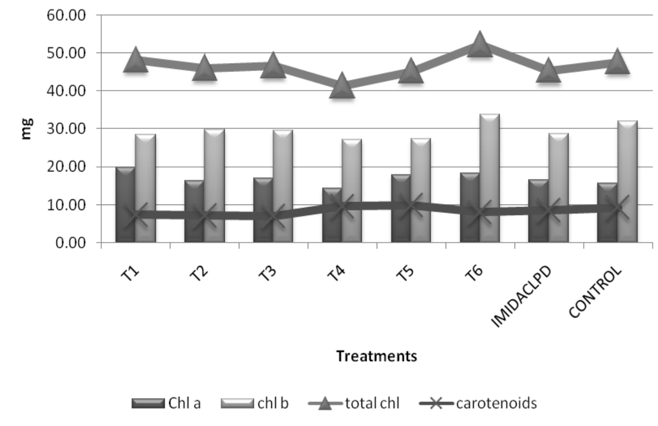

Figure 3. Chlorophyll and carotenoid content in infected leaves of turmeric plants treated with fungi against *U. folus*

No sign of deformity or mortality was observed in the first two days post treatment. Mortality began from day 3 and was high in about a week. The reason may be that infectious conidia may be produced only after few days after the spraying is done over the pest. Even though all stages are susceptible to fungal activity, it was observed that the stage of the larva plays an important part. In the present study, the third instar was affected more than the second and was susceptible to fungi. More investigation addressing the transmission mechanisms of the conidia is needed to characterize the patterns of the pest- fungi interaction.

Most of the biochemical parameters were humorously low in neem treated plots compares to other treatments. In general *T. viride* had the least effect on *U. folus*. The biochemical parameters tested in this treatments also revealed that the necessary nutrient were also minimally present in this treatment. The level of potassium was in considerable amount in all except first treatment with *M. anisopliae*. This is in accordance with the report of Sumathi *et al.* (2008) who assessed the influences of various biotic and abiotic factors in *Curcuma longa.* On the other hand, only a marginal difference was noticed with the chlorophyll and carotenoid content among the treatments 1, 2 and 3. The high yield of the PTS turmeric variety makes it a popular cultivar in South India. The mother rhizomes were used for the present study which gave good yields in almost all treated plots with a significant decrease in the control and neem treated plots which were susceptible more to pest attack. A similar observation was done by Kumar and Gill (2010).

In summary, the present study envisages the use of entomopathogenic fungi for pest control. *H. citriformis* showed good promise over *U. folus* and has practical application over the pest. This clearly indicated the potential of fungal antagonist in parasiting the pest. Further it has to be formulated suitably for local climatic conditions for long term use in turmeric cultivars.

ACKNOWLEDGEMENTS

The author is thankful to the Management and Principal of K.S.Rangasamy College of Technology, Tiruchengode for providing the infra structure facilities for the present study.

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Manuscript history

Received : 02.01.2012

Revised : 17.05.2012

Accepted : 20.05.2012