INTRODUCTION

Use of entomopathogenic fungi for biological control of insect pests is assuming increasing attention in recent times. It is believed that either fungal cells themselves or cell-free components would be equally effective. The first attempt to control a pest with a fungal agent was carried out in Russia in 1888, when the fungus now known as *Metarhizium anisopliae* (Metchn.) Sorokin was mass produced on beer mash and sprayed in the field for control of the beet weevil *Cleonus punctiventris* (Germar) (Lord, 2005). Deshpande (1999) studied the production of mycoinsecticides by Solid State Fermentation (SSF) and Submerged Fermentation (SMF), while Soccol et al., (1997) developed a SSF based process and investigated several agro-industrial substrates to produce spores from *Beauveria bassiana* for use in the biological control of pests of banana, sugarcane, soybean and coffee. Desgranges et al., (1993) produced *B. bassiana* spores by SSF for use against European corn borer. Statistical optimization of process variables has been reported for large scale production of *M. anisopliae* spores by SSF (Bhanu Prakash et al., 2008). The development and optimization of a simple production procedure will not only be valuable for the supply of reliable material for laboratory and field efficacy trials, but will also help to ensure the compatibility of the product with the formulation and application system.

Formulation technology is widely viewed as having great potential to improve the efficacy of microbial biocontrol agents (Burges 1998). The broad range of contemporary mycoinsecticide formulations, based on aqueous or oil carriers and incorporating surfactants, emulsifiers, and other adjuvants, have the potential to influence secondary dose acquisition. A number of laboratory and field studies indicate that oil formulation improves the efficacy of fungal pathogens (Inglis et al., 2002).

Oils and wetting agents have been extensively investigated and adopted as means of enhancing the delivery, persistence, and efficacy of mycoinsecticides. Oil based formulations of *Beauveria bassiana* (Balsamo) Vuillemin were introduced by Prior et al.,(1988), who reported efficacy of coconut oil for *B. bassiana* conidia rather than 0.01% aqueous Tween 80 for the weevil *Pantorhyzus politus* (Oberthür). Oil-based formulations of mycoinsecticides have been tested against various insect pests with positive results (Bateman et al., 1993; Filho et al., 1995; Inglis et al., 1996; Hidalgo et al., 1998; Legaspi et al., 2000; Malsam et al., 2002). Because of the lipophilic nature of phialoconidia that do not bear a mucus coating, they can be easily suspended in oils to achieve greater efficacies than when used in water (Bateman et al.,1993; David-Henriet et al.,1998). Cotton seed oil, soybean oil, and mineral oils reportedly do not adversely affect the viability of *B. bassiana* (Smart and Wright 1992, Grimm, 2001). Objectives of the present study were to test the unformulated and formulated conidiospores of *M. anisopliae* conidiospores during six months storage and to assess their viability as well as efficacy against *S. litura* in laboratory and field conditions.

MATERIALS AND METHODS

Preparation of infective propagules of *M. anisopliae*

*M. anisopliae* isolate M25 obtained from *Spodoptera litura* cadavers collected from the agricultural fields of Andhra pradesh, India was used for mass multiplication and formulation. Aerial conidia were produced using a standard two-phase production system (Jenkins et al., 1998; Bhanu Prakash et al., 2008), which consists of a submerged liquid culture followed by conidiation on a solid substrate. The submerged liquid culture comprise of simple liquid medium containing Dextrose-2g and peptone- 0.5g and 100 ml of distilled water to generate hyphal bodies and mycelium which forms inoculum for the second phase. Autoclaved grains of Barley/Sorghum/ Rice in polypropylene bags were used as solid substrates for the second phase. The bags were incubated for 15 days at 25±1°C for fungal development and subsequent sporulation. The substrate with conidia was transferred to plastic trays and kept for drying in the cabinet at 30±1°C. After two days when the moisture content was around 20%, conidia were separated by sieving through laboratory sieves of 1 mm mesh and further dried in an
auto-desiccator cabinet for four days to reduce the moisture content to 5%, because suitable optimal moisture content for long term dried conidia storage was found to be 4-5% (Moore et al., 1996).

**Conidial shelf life and viability at different temperatures**

The unformulated conidia were stored at different temperatures for a period of 6 months (4°C, -30°C and room temperature (29±1°C)). Conidial germination was tested at constant control temperature of 25±1°C. The germination assay was done with freshly isolated spores as control. An aqueous conidial suspension (100 μl containing 2.5 X 10^7 conidia/ml) was spread on SDAY medium and the inoculated Petri dishes in triplicate were incubated at 25±1°C in an environmental chamber. Plates were observed at 24 hourly intervals to test for germination. The count of germinated conidia was taken from 24h-96h after inoculation. Viability of conidia was tested at one month intervals for a period of 6month.

**Testing viability by laboratory bioassay against larvae of S. litura.**

Larvae of Spodoptera litura were obtained from the infested cauliflower fields of Visakhapatnam, Andhra Pradesh, India. A laboratory colony of S. litura originated from insect eggs was maintained at 25°C (8/16 h photoperiod) in plastic tubs. Nymphs and adults were reared on castor leaves.

Conidiospores harvested after drying at 30°C for 24 to 48h and stored at 4°C, -30°C and room temperatures for 15days, 2 months and 6months were used for pathogenicity studies on 3rd instar S. litura larvae. Conidial suspension in 0.02% Tween-80 (Sigma–Aldrich, India) was placed in a 50 ml glass vial with approximately 50 glass beads (3 mm diam.) and agitated for 5 min on a vortex mixture. Propagule concentrations were estimated using a hemocytometer and adjusted to 10^8 propagules ml^-1. Conidial viability was determined by plating preparations onto SDAY medium and examining colonies/ plate for each of the three replicates. An aliquot of the suspension was taken to check the viability of conidia as described by Varela and Morales (1996). The remaining suspension was stored in a refrigerator (4°C) for the treatments the next day. In a few samples, the viability was also tested after storing in the refrigerator. No significant variation in viability was observed in the aqueous conidial suspension tested before and after storing at 4°C.

Third instar S. litura larvae were chosen at random for each treatment batch, to test virulence of the fungal propagules stored at different temperatures. The larvae obtained from a single egg patch were used for each experiment. Larvae were resellected on the basis of size of the insect for homogeneity of the sample. Three replicates were maintained throughout the experiment. The insects were treated singly with 100μl of inoculum, dispensing with a micropipette (Gilson®) on the surface of the insect all over its body and head as per the method of Butt and Goettel (2000). S. litura larvae were placed in perforated plastic boxes (15 cm). Fresh castor leaves were provided as feed every day to the larvae, and the boxes were cleaned of insect litter daily. The insects were treated for 48h and the fatally injured larvac during inoculation were removed during treatment period. Mortality was recorded at 24h intervals till pupation. Controls were treated with an equal volume of 0.02% Tween 80 in sterile distilled water. Dead insects were immediately surface sterilized with 1% sodium hypochlorite followed by three rinses with sterile distilled water transferring to moist chambers (autoclaved Petri dishes with a moist filter paper lining) to facilitate mycosis. Pathogenicity experiments of S. litura against M. anisopliae were repeated twice. The pupae obtained from the remaining infected insects were kept in sterile moist sand for post pupation monitoring. The cumulative insect mortality in each treatment was corrected for control mortality (Abbott 1925). Median lethal time (LT₅₀) was calculated from the cumulative mortality data on each day post treatment, using probit analysis.

**Formulations**

Seven oil formulations and two powdered formulations were prepared with M. anisopliae conidiospores. One gram of Conidia from 2-week-old cultures grown on complete medium (SDAY) were collected aseptically and suspended in 2 ml of autoclaved and adequately cooled Peanut oil/ Gingelly oil/ Palm oil/ castor oil/ Sunflower oil/ Kerosene/ or Turpentine. For powder formulations, 1gm of dry conidial powder was mixed with 2gms of chalk powder/ talc powder in plastic vials. All the 7 formulations were stored at 4°C in refrigerator for 6 months.

**Laboratory bioassay of formulations against S. litura larvae.**

Laboratory evaluations of formulated M. anisopliae spores seven oil formulations and two powdered formulations were evaluated on 3rd instar S. litura larvae at 1st, 3rd and 6 months on gingelly oil, sunflower oil and peanut oil.

**Field application of unformulated and formulated conidiospores against S. litura larvae.**

Aerial conidia of an S. litura derived isolate of Metarhizium anisopliae, were mass multiplied on rice grains and harvested using a 1mm sieve (Bhanu prakash et al., 2008). The harvested conidia were dried to a water content of 5% at ambient temperature and suspended in 0.02% of liquid detergent solution.

The formulated spores of M. anisopliae showing 86% of germination after storage for three months at 4°C in gingelly oil were used for field evaluations. The emulsifiable formulation (EF) was prepared by mixing the oil formulation with 0.02% liquid detergent so as to get 1x10^{12} conidia/ml.

Vigna sinensis crop was raised in field plots of 2 sq. m. by maintaining 2 feet space within rows and 2 feet between rows. M. anisopliae conidiospores were used for spray application in the evenings between 16:00 h and 18:30 h. Field trials were conducted on bean crop (Vigna sinensis) infested with Spodoptera litura. All the larval stages of the pest were present on the crop at the time of application. Pest
load was noted before application of spores from randomly
selected areas in the field, which were covered and
protected using nylon net to avoid escape of pest from the
plants. Control plots were sprayed with liquid detergent
mixed water. First trial of mycopesticide application was
conducted during winters of two successive years.

**RESULTS**

*Shell life of infective propagules*

Shell life of the unformulated spores stored at
4°C, 30°C and room temperature for six months revealed
stability at -30°C after six months storage. Spores collected
from sorghum and barley substrates revealed better viability
after six months at -30°C (80%) in terms of germination
(Fig. 1). Samples stored at 4°C and 30°C showed better
viability than those stored at room temperature. Conidia
stored at room temperature displayed gradual decrease in
germination starting from 85% in the first month to 38% at
the sixth month. Conidia harvested from rice substrate
appeared to be drastically effected during storage based on
the drop in germination percent from 75% to 28% during
six months storage period.

*Viability testing by laboratory bioassay against 3rd instar S. litura larvace*

Effect of storage temperature and time on the
viurcellce of *M. anisopliae* towards *S. litura* indicated decrease
in insect mortality with increase in storage time (15day’s to
6months). Pathogenicity studies of the mass multiplied
conidia of *M. anisopliae* stored at the three temperatures on
the third instar larvae of *Spodoptera litura* at different time
intervals revealed drastic decrease in time of kill and LT50
values with respect to spores stored at room temperature
compared to the corresponding values of 15th day sample.

LT50 values of fresh spores on 15 th day of
harvesting ranged between 4.63 to 5.14 days (Table 1). Low
LT50 values were recorded for propagules produced on rice
(4.95). LT50 values of the spores stored for 6 months at
different temperatures was more compared to the value of
15th day sample. High LT50 values were recorded for
propagules obtained from rice substrate and stored at -30°C
(6.25) and on SDAY at 4°C (6.39). While the corresponding
values for spores produced on barley was 5.39 and 5.06 for
4°C and -30°C storage respectively. At room temperature
spores produced on sorghum showed lower LT50 values
than spores produced on barley. LT50 values of propagules
stored for six months at the same temperature revealed low
LT50 values for barley followed by sorghum at 4°C and
-30°C. At room temperature sorghum showed low LT50
value of 9.80 days and high LT50 value of 13.07 days on rice
substrate (Table 1).

*Viability testing by germination assay for formulated spores of M. anisopliae*  

Viability of conidia (expressed as percent
germination after 24h) in the formulations displayed
reduction over a period of six months (Fig. 2). Gingelly oil
and sunflower oil formulations showed better viability
compared to other formulations. Analysis of variance
indicated significant effect of isolate (P<0.001) and a
significant interaction between time and formulation
(<0.01). The overall decline of viable conidia was 13.33%
per month (calculated from the mean slope of regression).
Differences between the oil as well as powder formulations
were moderately less, though significant in some cases.
Difference in the mean values among the storage schedules

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**Table 1:** Laboratory bioassay data of mass multiplied dry spores of the entomopathogenic fungus *Metarhizium anisopliae* post 15th day, 2nd month and 6th month storage at 3rd instar *Spodoptera litura* larvae.

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
<th>Substrate</th>
<th>%Mortality</th>
<th>%Mycosis</th>
<th>LT50 (days)</th>
<th>Fiducial limits</th>
<th>RVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td>Room Tempera</td>
<td>Rice</td>
<td>86.67</td>
<td>57.69</td>
<td>5.14</td>
<td>4.76 – 5.54</td>
<td>0.181</td>
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<td></td>
<td>Room Tempera</td>
<td>Barley</td>
<td>88.33</td>
<td>54.72</td>
<td>4.63</td>
<td>4.69 – 5.42</td>
<td>1.378</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>Sorghum</td>
<td>86.67</td>
<td>59.62</td>
<td>5.08</td>
<td>4.72 – 5.45</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>Rice</td>
<td>86.67</td>
<td>57.69</td>
<td>4.95</td>
<td>4.58 – 5.31</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>SADAY</td>
<td>71.67</td>
<td>44.19</td>
<td>6.91</td>
<td>6.35 – 7.57</td>
<td>0.730</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>Rice</td>
<td>70.00</td>
<td>42.86</td>
<td>6.39</td>
<td>5.88 – 6.97</td>
<td>0.911</td>
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<tr>
<td></td>
<td>Room Tempera</td>
<td>SADAY</td>
<td>81.67</td>
<td>46.94</td>
<td>5.51</td>
<td>5.06 – 5.99</td>
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<td></td>
<td>Room Tempera</td>
<td>Sorghum</td>
<td>88.33</td>
<td>45.28</td>
<td>5.06</td>
<td>4.24 – 5.01</td>
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<td></td>
<td>Room Tempera</td>
<td>Rice</td>
<td>80.00</td>
<td>43.75</td>
<td>5.20</td>
<td>4.75 – 5.65</td>
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<tr>
<td></td>
<td>Room Tempera</td>
<td>SADAY</td>
<td>73.33</td>
<td>47.73</td>
<td>6.25</td>
<td>5.73 – 6.84</td>
<td>1.236</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>SADAY</td>
<td>70.00</td>
<td>40.48</td>
<td>7.43</td>
<td>6.79 – 8.24</td>
<td>0.320</td>
</tr>
<tr>
<td></td>
<td>Room Tempera</td>
<td>Rice</td>
<td>73.33</td>
<td>38.64</td>
<td>7.09</td>
<td>6.49 – 7.82</td>
<td>0.664</td>
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<tr>
<td>2 Months</td>
<td>-30°C</td>
<td>Sorghum</td>
<td>71.67</td>
<td>39.53</td>
<td>6.80</td>
<td>6.28 – 7.42</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>-30°C</td>
<td>Rice</td>
<td>58.33</td>
<td>34.29</td>
<td>9.16</td>
<td>8.26 – 10.47</td>
<td>1.485</td>
</tr>
<tr>
<td></td>
<td>-30°C</td>
<td>SADAY</td>
<td>55.00</td>
<td>30.30</td>
<td>9.52</td>
<td>8.55 – 10.94</td>
<td>0.537</td>
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<tr>
<td></td>
<td>-30°C</td>
<td>Rice</td>
<td>73.33</td>
<td>36.36</td>
<td>6.84</td>
<td>6.29 – 7.48</td>
<td>1.435</td>
</tr>
<tr>
<td></td>
<td>-30°C</td>
<td>SADAY</td>
<td>58.33</td>
<td>34.29</td>
<td>8.71</td>
<td>7.91 – 9.82</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>-30°C</td>
<td>Rice</td>
<td>53.33</td>
<td>28.13</td>
<td>9.27</td>
<td>8.40 – 10.51</td>
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<tr>
<td></td>
<td>-30°C</td>
<td>SADAY</td>
<td>71.67</td>
<td>34.88</td>
<td>6.65</td>
<td>6.11 – 7.27</td>
<td>0.050</td>
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<tr>
<td></td>
<td>-30°C</td>
<td>Rice</td>
<td>76.67</td>
<td>39.13</td>
<td>5.49</td>
<td>5.03 – 5.97</td>
<td>1.052</td>
</tr>
<tr>
<td></td>
<td>-30°C</td>
<td>SADAY</td>
<td>73.33</td>
<td>36.36</td>
<td>6.20</td>
<td>5.70 – 6.77</td>
<td>0.330</td>
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<tr>
<td></td>
<td>-30°C</td>
<td>Rice</td>
<td>75.00</td>
<td>28.89</td>
<td>5.97</td>
<td>5.50 – 6.48</td>
<td>1.334</td>
</tr>
<tr>
<td>6 Months</td>
<td>At 4°C</td>
<td>Sorghum</td>
<td>46.67</td>
<td>28.57</td>
<td>10.89</td>
<td>9.59 – 13.02</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>At 4°C</td>
<td>Rice</td>
<td>50.00</td>
<td>33.33</td>
<td>9.86</td>
<td>8.92 – 11.26</td>
<td>0.699</td>
</tr>
<tr>
<td></td>
<td>At 4°C</td>
<td>SADAY</td>
<td>51.67</td>
<td>32.26</td>
<td>9.80</td>
<td>8.78 – 11.34</td>
<td>0.810</td>
</tr>
<tr>
<td></td>
<td>At 4°C</td>
<td>Rice</td>
<td>40.00</td>
<td>25.00</td>
<td>13.07</td>
<td>11.17 – 6.63</td>
<td>1.353</td>
</tr>
</tbody>
</table>

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is greater than would be expected by chance after allowing for effects of differences in oil/powder and the values are statistically significant (P ≤ 0.05).

Viability testing of three of the oil formulations by bioassay against 3rd instar Spodoptera litura larvae.

Gingelly, sunflower and peanut oil formulations were tested at 1st, 3rd and 6th months of storage for viability by bioassay against 3rd instar larvae of Spodoptera litura. Gingelly oil formulation displayed maximum mortality of 86.33% of S. litura after one month storage and 73.33% after six months. The corresponding LT50 values were 4.33 and 6.89 days respectively (Table 2). On the other hand peanut oil formulation displaced least RVI value of -0.924 at 1st month compared to the value of 1.86 for gingelly oil formulation.

Field application

Field performance of fungal propagules was tested on Vigna sinensis crop heavily infested with S. litura. Data was taken at four random places in the field. Mortality of the pest was observed between fourth and eighth day after spraying. No mortality was detected in plots treated with distilled water + 0.02% liquid detergent which served as control. The mean percent larval mortality with respect to 2nd, 3rd, 4th and 5th instar larvae of S. litura was high in the application of formulation compared to application of unformulated spores (Fig. 2). Though maximum mortality (Fig. 3) of larvae at 2nd instar stage was recorded in the gingelly oil formulation as well as unformulated spore application, the values were superior in the gingelly oil (84.10%) compared to unformulated spores (74.65%).

Table 3: Mortality of S. litura on Vigna sinensis crop after application of formulated/unformulated spores of M. anisopliae.

<table>
<thead>
<tr>
<th>Instar</th>
<th>unformulated spores</th>
<th>with gingelly oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th day</td>
<td>8th day</td>
</tr>
<tr>
<td>2nd</td>
<td>34.65</td>
<td>74.65</td>
</tr>
<tr>
<td>3rd</td>
<td>21.64</td>
<td>55.86</td>
</tr>
<tr>
<td>4th</td>
<td>8.65</td>
<td>45.64</td>
</tr>
<tr>
<td>5th</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Laboratory bioassay data of formulated spores of the entomopathogenic fungus Metarhizium anisopliae post 1st, 3rd and 6th month against 3rd instar Spodoptera litura

<table>
<thead>
<tr>
<th>Time</th>
<th>Substrate</th>
<th>% Mortality</th>
<th>%Mycosis</th>
<th>LT50 (days)</th>
<th>Fiducial limits</th>
<th>RVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Month</td>
<td>Gingelly</td>
<td>88.33</td>
<td>84.91</td>
<td>4.33</td>
<td>3.91-4.73</td>
<td>1.062</td>
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<tr>
<td>3rd Month</td>
<td>Sunflower</td>
<td>81.67</td>
<td>81.63</td>
<td>5.12</td>
<td>4.63-5.61</td>
<td>-0.137</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>76.66</td>
<td>76.08</td>
<td>5.21</td>
<td>4.70-5.73</td>
<td>-0.924</td>
</tr>
<tr>
<td>6th Month</td>
<td>Gingelly</td>
<td>86.67</td>
<td>78.85</td>
<td>4.76</td>
<td>4.31-5.20</td>
<td>0.962</td>
</tr>
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<td></td>
<td>Sunflower</td>
<td>78.33</td>
<td>78.23</td>
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<td>4.80-5.90</td>
<td>0.072</td>
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<td>77.27</td>
<td>6.56</td>
<td>5.97-7.24</td>
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<td></td>
<td>Sunflower</td>
<td>73.33</td>
<td>79.55</td>
<td>6.89</td>
<td>6.26-7.65</td>
<td>0.963</td>
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<tr>
<td></td>
<td>Peanut</td>
<td>71.67</td>
<td>74.41</td>
<td>6.98</td>
<td>6.31-7.81</td>
<td>0.071</td>
</tr>
</tbody>
</table>

Fig. 1: Germination percentage of Metarhizium anisopliae dry spores over a period of six months storage at 4°C, -30°C and room temperature.

Fig. 2: Effect of different formulating agents on conidial viability (percent control values) of Metarhizium anisopliae over a six months period.

Fig. 3: Dead larvae of Spodoptera litura showing mycosis prior to sporulation on the eighth day of application using M. anisopliae formulation (infected larva of S. litura showing mycosis).
DISCUSSION

The experiment was conducted to assess the impact of storage temperatures on M. anisopliae mass multiplied spores and its infectivity against S. litura. The viability of conidia recorded a decline at room temperature and relative stability at -30°C. On the other hand Consolo et al., (2003) reported no difference in viability of the formulation of hyphomycetous fungi with vegetable oils (corn, sunflower and canola) at different temperatures (4, 17 and 26°C). Goettel et al., (1995) reported uniform germination of conidia of FHD13 isolate formulated in different vegetable oils and kerosene. On the other hand, Hidalgo et al., (1998) observed well maintained conidial viability in the fat pellet formulations and in the dustable powder (DP) but not so good in the oil suspension (OS) formulation. Luz and Batagin (2005) reported the influence of oils on the settling behaviour of Triatoma infestans nymphs and the activity of an oil–water formulation of the fungus against this vector under laboratory and simulated field conditions. The response of conidial longevity to temperature and humidity is predictable under consistent and fluctuating conditions (Hong et al., 1997 and 1999). Conidia can withstand high temperatures when dry and high humidity when cool, and different isolates can exhibit greatly different responses (Hong et al., 2001). Consequently, isolates could be selected on characteristics that include longevity under warm and humid conditions.

Shelf-life as well as biological and physical properties of the formulation are important issues in commercial formulations. Couch and Ignoffo (1981), suggested that formulations must remain stable for at least 1 year, but preferably for greater than 18 months for commercialization to occur. We found viability of around 40% of the conidia of M. anisopliae in the formulation after 6 months at room temperature. This decline in conidial viability of the fungus is acceptable in practical use or in the development of inert emulsions for crop protection purposes. Prior et al., (1988) suggested that oil-based formulations of B. bassiana have the advantage of allowing better adhesion of conidia to the hydrophobic cuticle of insects and reported better conidial survival in coconut oil than in water. Daoust et al., (1983) found that oils of different types, in a concentrated or pure form, have detrimental effect on M. anisopliae conidial viability during storage at certain temperatures, and relative humidity. Batta (2003) reported M. anisopliae formulation with coconut and soybean oils used in preparation of selected inert emulsion towards nymphs of the tobacco whitefly, Bemisia tabaci and the changes which occurs in the physical properties of these oils during rapid mechanical homogenization of emulsion ingredients.

In the field trials, gingly oil formulation of M. anisopliae applied at 10^{12} spores/ml showed effective control of 84.10% to 55.63% against the larval stages of S. litura completed to that of unformulated sample. Kaaya and Hassan (2000) found that mortality of unfed nymphal R. appendiculatus was greater when treated with oil-based than with aqueous formulations. Hornbostel et al., (2005) reported that in a field study, 10^3 spores/ml M. anisopliae did not effectively control questing I. scapularis nymphs, and significant differences were not detected in pre- and post-treatment densities. The enhanced efficacy of formulation is generally attributed to the fact that oils are excellent stickers, promoting contact between the formulated active ingredient and the lipophilic insect cuticle and increasing rain-fastness on the waxy leaf cuticle of treated host plants. The good pest control achieved in the field trial is a positive indication for inclusion of this fungus in the integrated pest management programmes.

The simple technology involved in the mass production of this easily cultured fungus makes it feasible for adoption by entrepreneurs interested in cottage industry. Insect全世界化 to the supply of formulation at a lower price as compared to the chemical pesticides. Further the method of broadcasting the fungus with the culture substrate is also feasible for promoting its further multiplication in the field, generating the high spore inoculum required for controlling the major leaf eating lepidopteran pests of economically important crops. Environmental safety and ecosystem stability considerations lead to the conclusion that the use of native isolates in a microbial control program is more convenient (Lockwood 1993). The practice of developing indigenous fungal strain is preferable for development of mycopesticide formulation for application against local pests in view of better ability to adapt to the local conditions.

REFERENCES

9. Daoust RA, Roberts DW, Studies on the prolonged storage of Metarhizium anisopliae conidia: effect of temperature and relative


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