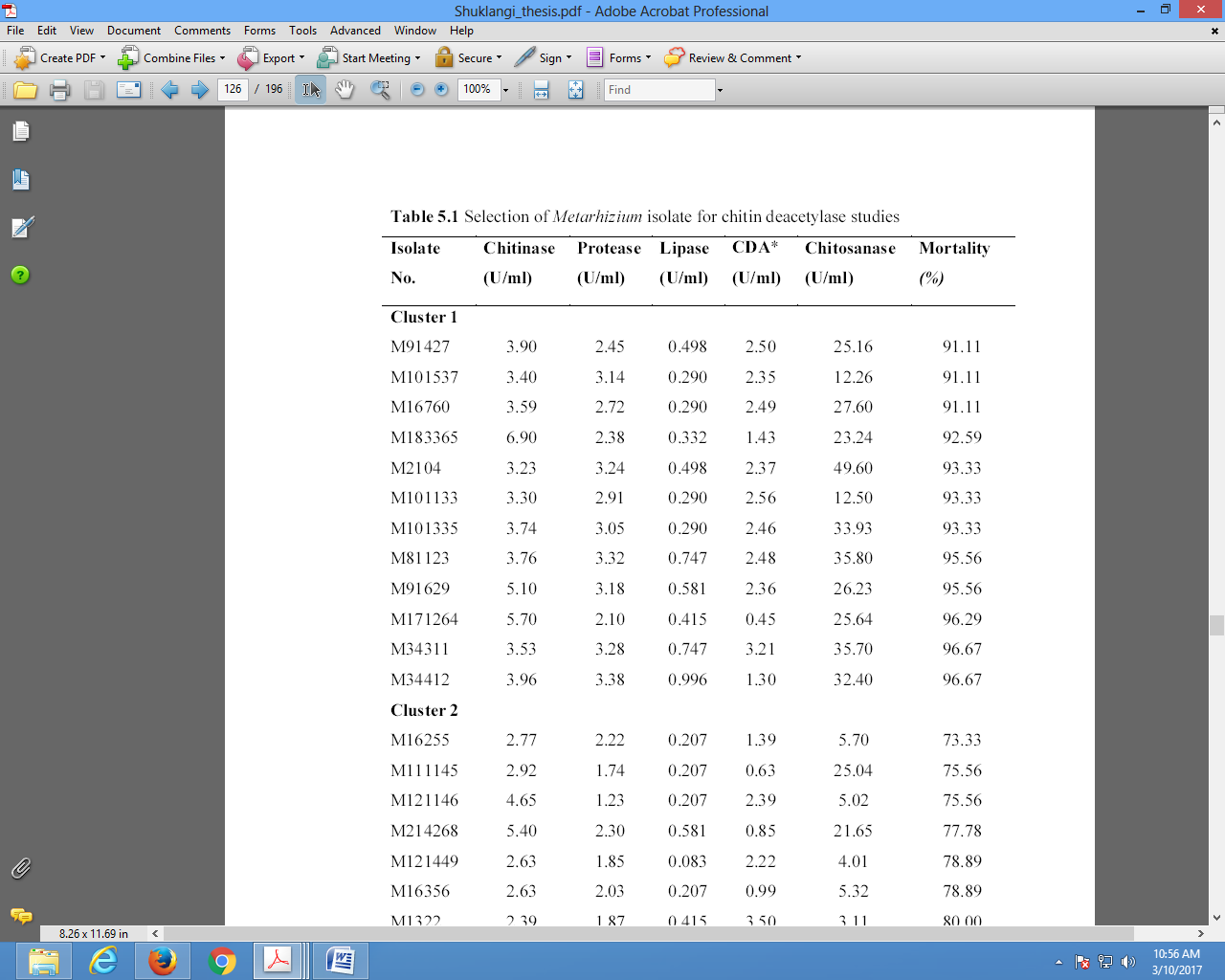
**Answer to the reviewer’s comment:**

*Reviewer #2:  
This study focused on isolation, bioassay, mass production and field trials of entomopathogenic fungi from various insects and soils. Although a lot of work was done, the data presented in Tables are of some technical problems. For instance, the authors claimed LC50 estimates of 1.4, 2.0 and 5.7 x 103 conidia for their selected isolates against the third instar larvae of Helicoverpa armigera but disclosed insufficient technical information. With my 30-year experience working on fungal insect pathogens, the LC50 estimates are too low to be true for fungal insect pathogens. Their multi-concentration bioassays were carried out by dipping the tested larvae in the 10^3, 10^5, 10^7 and10^9 spores/ml suspensions, which are technically questionable and should be changed to 10^4, 10^5, 10^6 and10^8 spores/ml for fungal infection through cuticular penetration. In another bioassay, the use of a 10^7 spores/ml suspension resulted in an LT50 of 3.3-3.5 days, which is also too good to be true. I guess that the authors could encounter a problem in quantifying precisely the concentration of original spore suspension. The results from field trials are encouraging but no details are disclosed on how the field trials were designed and performed. Nor were standard deviations or errors associated with pod damage percentage and pod yield, which should be statistically analyzed. The manuscript was written in a way to make it far away from a scientific report for a journal. I am not sure of that the writing format is acceptable for the e-journal.***Your Response:**  
*The data presented in the present m/s is by using first subculture from the mycosed larva of H. armigera (Ref.15). However, as rightly pointed out, after repeated subuculturing on artificial medium the LC50 for M34412 was 3 x 104/ml and LT50 was increased to 5.6 days. In Table 2, the fiducial limits for LC50, LT50 and ST50 have been given (Ref. 8). In the revised m/s it has been indicated on line 158 (3.2.1) that for insect bioassay, production of spores and field performance studies, the first subcultures of Metarhizium strains from mycosed H. armigera larvae were used, unless otherwise mentioned.*

Appreciating expertise of the reviewers and their concern (which now duly acknowledged in the acknowledgement), I would like to add more details regarding our data along with some observations:

I have checked the values presented in Table 2 and all the values are correct and are from published work. I am also adding one more reference of a PhD thesis which is available online for your perusal. The counting of the conidia is routinely done using haemocytometer and it has been done by different students to cross check the values. In my opinion, it is unlikely that there is an error in counting.

We started this project with isolation of different entomopathogenic fungi and we collected *Beauveria* and *Nomuraea* strains also. As conidia are comparatively dry, the yield of conidia is high in solid state fermentation for *Metarhizium* isolates we further tested 68 *Metarhizium* isolates from soil samples from different host and non host crops for *H. armigera*  as well as from different insects. From 68, we selected 12 best isolates which were highly virulent in laboratory bioassay (Table below from Kulkarni S.A. (2015)):



The table is from: Kulkarni S.A. (2015) Biochemical and molecular studies of chitin deacetylase from *Metarhizium* species. A PhD thesis submitted to Savitribai Phule Pune University, Pune, India. Available on: <http://ncl.csircentral.net/1696/1/Kulkarni%20Shuklangi_Ph.D.%20thesis.pdf>

From this, based on LC50, LT50, ST50, yield and other parameterswe selected 3 best performers. We can give in detail technical information for all the experiments. As the number of references is restricted we have not given all the references of our published work. I am attaching the list of references published on this work for your ready reference.

It has been published that the LC50 value of the 40th sub-culture increased as compared to that of the first sub-culture (Nahar et al, 2008). This will affect the field performance of the 40th sub-culture. The LC50 can be decreased by passaging *M. anisopliae* through *H. armigera*. Alternatively, the same level of efficacy can be obtained by applying > 20% conidial inoculum under field conditions. However, the selection of either of the above strategies would greatly depend on the cost factor. Similarly it has been suggested that LT50 can also be useful to reduce cost as this will not allow larvae to enter in to further in star which could become more resistant.

For example, the virulence of *Paecilomyces farinosus* towards the English grain aphid, *Sitobion*

*avenae* (F.) increased after host passage (Hayden et al. 1992). After three selective sub-cultures of *P. farinosus*, LT50 decreased from 11 to 5 days. The changes in the LT50 of the present strain *M. anisopliae* after passage through *H. armigera* were in accordance with the earlier reports (Nahar et al, 2008).

The significance of these parameters, especially LC50 and LT50 was highlighted in the co-development programme by industry partner for cost reduction. In the solid state fermentation, 70 g (4 x 1010 conidia/ g) conidia/ kg of rice are produced. For 1 hectre field to control *H. armigera* in pulses, usually 1 x 1012 conidia are sprayed 3 times with 15 days interval, which comes down to 120 g conidia in formulation. The production of conidia cannot be increased beyond certain limit as there is a delicate balance between number and virulence. Under the circumstances, it is possible that strain which has lower LC50 and LT50 can be used in lower number in the field, which in turn be useful to reduce the cost, which is one of the challenges ahead (Kapoor and Deshpande, 2013). At present, we are working in this direction too.

**Insect bioassay (LC50)**

The insect bioassays were carried out using 3rd instar larvae of *H. armigera*. The set of 30 larvae in triplicate were dipped individually in 10 ml conidial suspension of *Metarhizium* isolates for 5 sec. After treatment, each larva was individually transferred to a separate sterile vial containing moist Whatmann filter paper No. 1 and a piece of disinfected okra that was changed on alternate days. The larvae were kept at 25±2°C, 65±5% RH and 16:8 (L: D) for 14 d or until they died. To allow mycelia and conidia formation over the cadavers, the dead larvae were transferred to sterile petri plates containing moist cotton swabs and kept at 28°C and 70-80% RH for at least 3-7 d. A set of 30 larvae in triplicate treated with 0.1% (w/v) Tween 80 in sterile distilled

water served as a control. The experiment was conducted in triplicate using freshly prepared conidial suspensions. The data on percent mortality from three experiments were pooled to get average values, which were corrected by Abbott’s formula (Abbott, 1925).

The median lethal concentration (LC50) of *Metarhizium* isolates against 3rd instar larvae of *H. armigera* was determined to increase the possibility of identifying the difference in virulence of isolates with high mortality values that might go undetected if only a single dose was used. The LC50 values were determined using four concentrations (1×103, 1×105, 1×107 and 1×109 conidia/ml) of conidial suspension. The experimental layout was a RCBD with each treatment containing a set of 30 larvae repeated 3 times.

The field trials were performed with Agriculture University and the results were obtained from them officially, which is the requirement for Central Insecticide Board, India registration. The farmers’ participatory trial was for demonstration organized by the Agriculture University. Though we participated in the trials the data was authenticated by Director of Research, (DOR) Agriculture University (MPKV, Rahuri). The values of standard deviation for percent pod damage as per report are included in Table-3.

**List of publications on this topic:**

**Research papers**

1. Vidhate R., Singh J., Ghormade V., Chavan S.B., Patil A. and **Deshpande M.V.** (2015) Use of Hydrolytic enzymes of *Myrothecium verrucaria* and conidia of *Metarhizium anisopliae*, singly and sequentially to control pest and pathogens in grapes and their compatibility with pesticides used in the field. Biopestic. Int **11**: 48-60.

1. Kapoor M., Pawar P.V., Joseph M., Sen A. and Deshpande M.V. (2013) Evaluation of biocontrol potential of *Metarhizium anisopliae* strains against larvae and adults of *Aedes aegypti* (L.). J. Biol. Control **2**7: 194-203
2. Yadav P and **Deshpande M.V**. (2012) Control of beet armyworm, *Spodoptera litura* (Fabricius) by entomopathogenic fungi, *Nomuraea rileyi* N812, *Beauveria bassiana* B3301 and *Metarhizium anisopliae* M34412. Biopestic. Int. **8**: 107-114
3. Kulkarni S.A., Ghormade V., Kulkarni G., Kapoor M, Chavan S.B., Rajendran A., Patil S.K., ShoucheY., and **Deshpande M.V**. (2008)Comparison of *Metarhizium* isolates for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. Biocontrol Sci. Technol. **18**: 809-828. (IF 1.087; 2)
4. Nahar P.B., KulkarniS.A., Kulye M.S., ChavanS.B.,KulkarniG., RajendranA.,YadavP.D., ShoucheY. and **Deshpande M.V.** (2008) Effect of repeated *in vitro* sub-culturing on the virulence of *Metarhizium anisopliae* against *Helicoverpa armigera* (Lepidoptera: Noctuidae). Biocontrol Sci. Technol.**18**:337-355 (IF 1.087; 5)
5. Nahar P.B., Ghormade V. and **Deshpande M.V**. (2004) The extracellular constitutive production of chitin deacetylase in *Metarhizium ansiopliae*: Possible edge to entomo-pathogenic fungi in the bio-control of insect pest. J. Invertebr. Pathol*.* **85**: 80-88 (IF 1.060; 28) **(Featured in Newsindia, a Nature's science & policy round-up for India, June 2004).**
6. Nahar P.B., Kulye M., Yadav P., Hassani M., Tuor U., Keller S. and **Deshpande M.V.** (2003) Comparative evaluation of indigenous fungal isolates, *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N812 for the control of *Helicoverpa armigera* (Hüb.) on chickpea. Indian J. Mycol. Plant Pathol*.* **33**: 372-377
7. Nahar P.B., Yadav P., Kulye M., Hadapad A., Hassani M., Tuor U., Keller S. Chandele A., Thomas B. and **Deshpande M.V.** (2004) Evaluation of indigenous fungal isolates, *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N812 for the control of *Helicoverpa armigera* (Hübner) in pigeon pea field. J. Biol. Control **18**:1-7

**Reviews**

1. Vidhate R., Ghormade V., Kulkarni S., Mane S., Chavan P. and **Deshpande M.V.** (2013) Mission Mode Collections of Fungi with Special Reference to Entomopathogens and

Mycopathogens. Kavaka 41:33-42

1. Kapoor M. and **Deshpande M.V.** (2013) Development of mycoinsecticide for the control of insect pests: Issues and challenges in transfer of technology from laboratory to field. Kavaka 40: 45-56
2. Yadav P and **Deshpande M.V.** (2010) Fungus- fungus and fungus–insect interactions. Biopestic. Int. **6**:21-35

1. **Deshpande M.V**. (1999) Mycopesticide production by fermentation: Potential and challenges. Critical Reviews in Microbiology **25**: 229-243. (IF 1.917; 26)

**Chapters**

1. Chavan S., Kulkarni M. and **Deshpande M. V**.(2008) Status of microbial pesticides in India In: Review of Plant Pathology Vol. 4 (Ed. Prof. S. M. Reddy and H.N. Gour) pp.393-420, Sci. Publishers, Jodhpur
2. Chavan S, Ghormade V, Nahar P, and **Deshpande MV.** (2006) Entomopathogenic fungi: A valuable tool to fight against insect pests. In: Plant Protection for the New

Millennium.Vol.II eds. AV Gadewar, and BP Singh, pp. 227-243 Delhi: Satish Serial

Publishing House.

1. **Deshpande M.V.** (2005) Formulations and applications of mycopathogens. In: Microbial biopesticide formulations and application. Tech.Document No. 55. Eds. R.J.Rabindra, S.S.Hussaini and B.Ramanujam. pp.150-158, PDBC, Bangalore.
2. **Deshpande M.V**. (2005) Mycopesticides: Their potential and challenges In : Fungi:Diversity and Biotechnology*.* Ed. M.K.Rai and S.K.Deshmukh, pp.375-390. Scientific Publishers, Rajasthan.
3. Hassani M., Tuor U., **Deshpande M**., Hadapad A., Nahar P., Bucher T., Kulye M., Yadav P., Enkerli J. and Keller S. (2004) Mass production of entomogenous fungus Metarhizium anisopliae (Deuteromycotina: Hyphomycetes) for biological control of helicoverpa armigera (Lepidoptera: Noctuidae)on pulses. In: Proceeding of the international workshop on entomopathogenic fungi - a valuable alternative to fight against insect pests ( September 16-18, 2002). Ed. M.V.Deshpande, pp. 37-49, National Chemical Laboratory, Pune, India.
4. **Deshpande M.V**., Keller S., Hassani M., Chandele A., Nahar P., Hadapad A., Kulye M., Yadav P. Bucher T. and Tuor U. (2004) Comparative evaluation of indigenous fungal isolates , *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi*  N812 for the control of *Helicoverpa armigera* (Hüb.) on pulses. In: Proceeding of the international workshop on entomopathogenic fungi - a valuable alternative to fight against insect pests ( September 16-18, 2002). Ed. M.V.Deshpande, pp. 51-59, National Chemical Laboratory, Pune, India.
5. **Deshpande M.V.,** Chandele A.G., Nahar P. , Hadapad A. , Patil, G., Ghormade V., Keller S. , and Tuor U. (2003) Entomopathogenic fungi: Mycoinsecticides useful against lepidopteran pest in pulses. IOBC/WPRS Bull. **26**: 27-30.
6. **Deshpande M.V.** and Tuor U. (2001) Microbial control of pests: Entomopathogenic fungi as

mycoinsecticides. Proceedings of the III Asia Pacific Crop Protection Conference-2001. Pesticides Manufacturers & Formulators Association of India, Mumbai. pp. 56-59.

1. **Deshpande M.V.** (2000) Mycopesticide production: Contribution of fungal morphologies in the biopesticide formulations. Nat. Bot. Soc. 55:11-17 [Peer Reviewed].
2. **Deshpande M.V.** (1999) Mycopesticide production: Potential and challenges. In: Proc. National Seminar on Advances in Plant Pathology, University of Pune, Pune. pp. 21-30.
3. **Deshpande M.V**. (1998) Biopesticide production by fermentation: Scope and limitations.

In: Microbial Pesticides & Insect Pest Management (H.D.Rananavare, S.R.Naik and T.K.Dongre, eds) 75-80, BARC, Mumbai and HAL, Pune.