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In-vitro evaluation of wettable powder and liquid formulations of *Metarhizium anisopliae* against Tobacco leaf eating caterpillar, *Spodoptera litura* fab. (Noctuidae: Lepidoptera)

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Abstract
Spodoptera litura is a serious cosmopolitan pest with a wide host range of field as well as horticultural crops. *In-vitro* experiments were conducted at Department of entomology, Annamalai university to evaluate the efficacy of different concentration of commercial wettable powder and liquid formulations of entomopathogenic fungi, *Metarhizium anisopliae* against *S.litura* by spray and leaf-dip method. Percentage of larval,pupal,adult mortality, pupal formation and adult emergence were evaluated.The results revealed that liquid formulation of *M.anisopliae* @ 1×10^{12} spores/ml by spray method resulted in higher larval mortality (52.50%), pupal and adult mortality (35%,12.50%), lower pupal formation and adult emergence (45.50%,12.50%) when compared to wettable powder formulations by spray& leaf dip method and liquid formulations by leaf dip method.

Keywords: *Metarhizium anisopliae, Spodoptera litura,* liquid, wettable powder formulations

Introduction

The tobacco leaf eating caterpillar, *Spodoptera litura* Fab. is a serious polyphagus noctuid pest which has constant association with many agricultural crops causing losses to pulses, oilseeds, vegetables *etc*. Indiscriminate use of chemical pesticides has not only resulted in various environmental hazards but also development of insecticide resistance in insect pests. This resistance exhibited by *S. litura* has led to sporadic outbreaks and crop failure (Ahmad *et al*. 2005) [1]. Use of entomopathogens for pest management is one of the important components in Integrated Pest Management (IPM). Among entomopathogens, entomopathogenic fungi are considered as a good alternative for pest management, as they are eco-friendly and non-toxic to non-target oraganisms and they also occupy predominant position among bio control agents (Karthikeyan, 2012) [10]. Some major entomopathogenic fungi which are employed in pest management are *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, *Verticilium lecanii.* Among the entomopathogenic fungi, the green muscardine fungus is found to be effective against *Spodoptera litura* (Dayakar and Kannauja, 2003) [5]. Because of its green colour sporulating colonies, *M. anisopliae* is characterised as “green muscardine fungus”. Nahar *et al.* (2004) [13] reported the virulence and effectiveness of *M. anisopliae* against *S. litura*. *M. anisopliae* forms a mycelial mat on insect’s cuticle, infective unit known as conidia geriminates and forms a germ tube which bears appressoria with infective peg attached to insect’s cuticle. By the process of enzymatic dissolution of chitin and protein, these infective pegs penetrate the insect’s integument layer and reaches haemocoel and internal organs and insect body is filled with fungus. Symptoms exhibited by infected insects are loss of appetite, loss of mobility, discolouration and mummification (Indrayanti, 2017) [8].

Materials and Methods

The Present research studies entitled as *In-vitro* evaluation of wettable powder and liquid formulations of *Metarhizium anisopliae* against Tobacco leaf eating caterpillar, *Spodoptera litura* fab. (Noctuidae: Lepidoptera) was carried out in Bio-control laboratory, Department of Entomology, Annamalai university, Chidambaram, Tamil Nadu, India.

Culturing of test insect, *Spodoptera litura*

The egg masses of tobacco caterpillar, *Spodoptera litura* were collected from black gram field at Vallampadugai, Cuddalore District, Tamil Nadu. The surface of egg masses were sterilized with 0.02% of sodium hypochlorite solution and allowed to hatch. After hatching, the neonate larvae were transferred to a tray containing castor leaves. The tray is covered with cauda cloth with thread tied over the tray. Castor leaves were provided as feed for *Spodoptera litura* till the pre-pupal stage. Then, sterilized soil is provided for pupation. After pupation, the pupae were collected and transferred to the oviposition chambers (40×5×5 cm) for adult emergence. After the emergence of adults, cotton plug soaked with 10% (w/v) sugar solution and few drops of multi vitamin were provided to adults in order to increase the fecundity. Nerium leaves were kept inside the adult emergence cage for egg laying. The adults of *Spodoptera litura*, layed the eggs on undersurface of nerium leaves. Then the eggs were collected and allowed for hatching. After hatching, the neonate larvae were provided with tender castor leaves as feed. These laboratory reared larvae of *Spodoptera litura* were used for bio assay, at room temperature (7 ± 2^0 c) with 14-10 light dark photoperiod and 75 ± 5% relative humidity.

Preparation of Entomopathogenic fungi, *Metarhizium anisopliae*

Commercial wettable powder and liquid formulations of *Metarhizium anisopliae* were used for this evaluation against *S. litura*. Different concentrations of *M. anisopliae* were prepared using serial dilution for bioassay by 2 methods: Spray and leaf-dip method.

Spray Method

Fresh castor leaf discs of 6cm size are placed in sterile petridish with moist tissue paper placed over it. Four newly moulted second instar larvae were released in each petridish. Five different concentrations of wettable powder and liquid formulations of *M. anisopliae* were prepared (1×10^4,1×10^6,1×10^8,1×10^{10} and 1×10^{12} spores/ml) and were directly sprayed on the second instar larvae *S. litura* using a hand atomizer. Each treatment is replicated four times in each experiment. Water spray served as a control. The larval mortality percentage, pupal formation, pupal moratlity percentage and adult emergence percentage were calculated and results were tabulated.

Leafdip Method

Fresh castor leaves were collected from an unsprayed castor field and washed in running water to remove contaminants before being cut into 6cm discs. Then, the leaf discs were dipped on different spore suspensions (1×10^4, 1×10^6, 1×10^8, 1×10^{10} and 1×10^{12} spores/ml) of both wettable and liquid formulations of *M. anisopliae* prepared by serial dilution. After dipping, castor leaves were allowed to dry and placed in petriplates containing moist tissue paper to provide humidity. Then, 4 larvae were released in each petriplates. Leaf discs were replaced at an interval of 2-3days. Each treatment is replicated four times in each experiment. The larval mortality percentage, pupal percentage, pupal moratlity percentage and adult emergence percentage were calculated and results were tabulated.

Statistical analysis

In-vitro experiments were conducted in the CRBD design with required replications (Panse and Sukhatme,1978). Per cent were arc sine transformed and then analyzed. The obtained data were analyzed by using computer based OPSTAT analysis developed by, CCS HAU, Hisar, Haryana.

Results

In Spray method, among the different concentrations of wettable powder formulations of *M.anisopliae*, the highest larval mortality (37.5%), lowest pupa formation (62.5%), pupal mortality (36.66%), lowest adult emergence (26.66%) and adult mortality (23.33%) was observed in *M. anisopliae* @ 1×10^{12} spores/ml. This was followed by *M. anisopliae* @ 1×10^{10} spores/ml, 1×10^8 spores/ml, 1×10^6 spores/ml which resulted in larval mortality (25%, 12.5% and 10%), pupa formation (75%, 87.5% and 90%), pupal mortality (33.33%, 23.33% and 13.33%), adult emergence (43.33%, 66.66% and 76.66%) and adult mortality (20%, 16.66% and 13.33%). Lowest larval mortality (5%), highest pupa formation (95%), lowest pupal mortality (10%), highest adult emergence (86.66%) and adult mortality (10%) was observed in *M. anisopliae* @ 1×10^4 spores/ml (TABLE-1). Among the different concentrations of liquid formulations of *M. anisopliae*, the highest larval mortality (52.5%), lowest pupa formation (47.5%), highest pupal mortality (35%), lowest adult emergence (12.5%) and highest adult mortality (22.5%) was observed in *M. anisopliae* @ 1×10^{12} spores/ml. This was followed by *M. anisopliae* @ 1×10^{10} spores/ml, 1×10^8 spores/ml, 1×10^6 spores/ml which resulted in larval mortality (37.5%, 25% and 20%), pupa formation (62.5%, 75% and 80%), pupal mortality (27.5%, 22.5% and 17.5%), adult emergence (35%, 52.5% and 62.5%) and adult mortality (17.5%, 15% and 7.5%). Lowest larval mortality (12.5%), highest pupa formation (87.5%), lowest pupal mortality (15%), highest adult emergence (72.5%) and lowest adult mortality (5%) were observed in *M. anisopliae* @ 1×10^4 spores/ml (TABLE-2).

In leaf dip method, among the different concentrations of wettable powder formulations of *M. anisopliae*,the highest larval mortality (25%), lowest pupa formation(75%), pupal mortality (20%), adult emergence (55%) and adult mortality (22.5%) was observed in *M. anisopliae* @ 1×10^{12} spores/ml. This was followed by *M. anisopliae* @ 1×10^{10} spores/ml, 1×10^8 spores/ml, 1×10^6 spores/ml which resulted in larval mortality (17.5%, 10 % and 7.5%), pupa formation (82.5%, 90% and 92.5%), pupal mortality (17.5%, 12.5% and 10%), adult emergence (65%, 77.5% and 80%) and adult mortality (15%, 10% and 7.5%). Lowest larval mortality (2.5%), highest pupa formation (95%), lowest pupal mortality (7.5%), highest adult emergence (87.5%) and lowest adult mortality (5%) was observed in *M. anisopliae* @ 1×10^4 spores/ml (TABLE-3).Among the different concentrations of liquid formulations of *M. anisopliae*, the highest larval mortality (45%), lowest pupa formation (90%), highest pupal mortality (27.5%), lowest adult emergence (27.5%) and highest adult mortality (12.5%) was observed in *M. anisopliae* @ 1×10^{12} spores/ml. This was followed by *M. anisopliae* @ 1×10^{10} spores/ml, 1×10^8 spores/ml, 1×10^6 spores/ml which resulted in larval mortality (35%, 30% and 17.5%), pupa formation (65%, 70% and 82.5%), pupal mortality (20%, 17.5% and 15%), adult emergence (45%, 52.5% and 67.5%) and adult mortality (10%, 7.5% and 5%). Lowest larval mortality (10%), highest pupa formation (90%), lowest pupal mortality (12.5), highest adult emergence (77.5%) and lowest adult mortality (2.5%) were observed in *M. anisopliae* @ 1×10^4 spores/ml (TABLE-4).

Table 1: Effect of wettable powder formulations of *M.anisopliae* against the biology of *S.litura* by spray method

S. No	Treatments (Spores/ml)	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	1×10^4	5.00 (9.21) ^{e}	95.00 (80.76) ^{b}	10.00 (18.42) ^{d}	86.66 (67.47) ^{b}	10.00 (18.42) ^{d}
2	1×10^6	10.00 (18.42) ^{d}	90.00 (71.53) ^{c}	13.33 (22.49) ^{c}	76.66 (60.08) ^{c}	13.33 (20.45) ^{c}
3	1×10^8	12.50 (20.45) ^{c}	87.50 (69.50) ^{d}	23.33 (29.50) ^{b}	66.66 (52.47) ^{d}	16.66 (24.52) ^{b}
4	1×10^{10}	25.00 (29.87) ^{b}	75.00 (60.08) ^{e}	33.33 (36.20) ^{a}	43.33 (39.15) ^{e}	20.00 (24.15) ^{b}
5	1×10^{12}	37.50 (37.71) ^{a}	62.50 (52.25) ^{f}	36.66 (35.11) ^{a}	26.66 (29.72) ^{f}	23.33 (28.21) ^{a}
6	Control	0.00 (0.00) ^{f}	100.00 (90.00) ^{a}	0.00 (0.00) ^{e}	100.00 (90.00) ^{a}	0.00 (0.00) ^{e}
CD (p =0.05)		7.57	7.58	6.04	7.74	5.96
SE (d)		3.57	3.58	2.85	3.65	2.81

Each value is mean of four replications

Figures in parentheses are arc sine transformed values

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 2: Effect of liquid formulations of *M. anisopliae* against the biology of *S. litura* by spray method

S.No	Treatments (Spores/ml)	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	1 ×10^4	12.50 (20.45) ^{e}	87.50 (69.50) ^{b}	15.00 (22.49) ^{e}	72.50 (58.42) ^{b}	2.50 (4.60) ^{e}
2	1 ×10^6	20.00 (26.55) ^{d}	80.00 (63.40) ^{c}	17.50 (24.52) ^{d}	62.50 (52.25) ^{c}	5.00 (9.21) ^{d}
3	1 ×10^8	25.00 (29.87) ^{c}	75.00 (60.08) ^{d}	22.50 (28.21) ^{c}	52.50 (46.42) ^{d}	7.50 (13.82) ^{c}
4	1 ×10^{10}	37.50 (37.71) ^{b}	62.50 (52.31) ^{e}	27.50 (31.53) ^{b}	35.00 (36.20) ^{e}	10.00 (18.42) ^{b}
5	1 ×10^{12}	52.50 (46.42) ^{a}	47.50 (43.54) ^{f}	35.00 (36.20) ^{a}	12.50 (20.45) ^{f}	12.50 (20.45) ^{a}
6	Control	0.00 (0.00) ^{f}	100.00 (90.00) ^{a}	0.00 (0.00) ^{f}	100.00 (90.00) ^{a}	0.00 (0.00) ^{f}
C.D (p =0.05)		4.26	5.16	5.21	4.61	10.57
SE (d)		2.01	2.44	2.46	2.17	4.99

Each value is mean of four replications
Figures in parentheses are arc sine transformed values
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 3: Effect of wettable formulations of *M.anisopliae* against the biology of *S. litura* by leaf dip method

S.No	Treatments (Spores/ml)	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	1 ×10^4	2.50 (4.60) ^{e}	95.00 (80.76) ^{b}	7.50 (13.82) ^{e}	87.50 (69.50) ^{b}	5.00 (9.21) ^{e}
2	1 ×10^6	7.50 (13.82) ^{d}	92.50 (76.15) ^{c}	10.00 (18.42) ^{d}	80.00 (63.40) ^{c}	7.50 (13.82) ^{d}
3	1 ×10^8	10.00 (18.42) ^{c}	90.00 (74.12) ^{d}	12.50 (20.45) ^{c}	77.50 (62.12) ^{d}	10.00 (18.42) ^{c}
4	1 ×10^{10}	17.5 (24.52) ^{b}	82.50 (65.44) ^{e}	17.50 (24.52) ^{b}	65.00 (53.91) ^{e}	15.00 (22.49) ^{b}
5	1 ×10^{12}	25.00 (29.87) ^{a}	75.00 (63.57) ^{f}	20.00 (26.55) ^{a}	55.00 (48.14) ^{f}	22.50 (28.21) ^{a}
6	Control	0.00 (0.00) ^{f}	90.00 (90.00) ^{a}	0.00 (0.00) ^{f}	100 (90.00) ^{a}	0.00 (0.00) ^{f}
C.D(p =0.05)		8.66	15.12	6.63	8.99	9.29
SE (d)		4.09	7.42	3.13	4.25	4.38

Each value is mean of four replications
Figures in parentheses are arc sine transformed values
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 4: Effect of liquid formulations of *M.anisopliae* against the biology of *S.litura* by leaf dip method

S.No	Treatments (Spores/ml)	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	1× 10^4	10.00 (18.42) ^{e}	90.00 (71.53) ^{b}	12.50 (20.45) ^{d}	77.50 (61.74) ^{b}	5.00 (9.21) ^{e}
2	1 ×10^6	17.50 (24.52) ^{d}	82.50 (64.44) ^{c}	15.00 (24.52) ^{c}	67.50 (55.26) ^{c}	7.50 (13.82) ^{d}
3	1 ×10^8	30.00 (33.19) ^{c}	70.00 (56.76) ^{d}	17.50 (24.52) ^{c}	52.50 (46.42) ^{d}	15.00 (22.49) ^{c}
4	1 ×10^{10}	35.00 (36.20) b	65.00 (53.75) ^{e}	20.00 (26.18) b	45.00 (42.09) ^{e}	17.50 (24.56) ^{b}
5	1 ×10^{12}	45.00 (42.09) ^{a}	55.00 (42.09) ^{f}	27.50 (31.53) ^{a}	27.50 (31.53) ^{f}	22.50 (28.21) ^{a}
6	Control	0.00 (0.00)	100.00 (90.00) ^{a}	0.00 (0.00) ^{e}	100.00 (90.00) ^{a}	0.00 (0.00) ^{f}
CD (p =0.05)		3.84	3.84	6.02	4.34	9.61
SE (d)		1.81	1.81	2.84	2.05	4.54

Each value is mean of four replications
Figures in parentheses are arc sine transformed values
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Discussion

In a similar study, Hussain *et al*. (2011) [7] reported the liquid suspensions of *M. anisopliae* at higher spore concentrations resulted in maximum mortality of *Microtermesobesus*, *Odontotermes obesus* under laboratory conditions. Asi *et al*. (2013) [2] stated that *M. anisopliae* @1×10^8 spores/ml found to be effective against eggs, larvae and pupae of *Spodoptera litura*. Also in a similar study, Halawa *et al*. (2019) [6] reported that early instar larvae is more susceptible to *M. anisopliae* than late instar larvae of *Spodoptera litura*. Rivera *et al*. (2014) [15] stated the commercial strains of *M. anisopliae* resulted in higher larval mortality, pupal mortality, lowest adult emergence and higher adult malformation at higher spore concentration (53×10^4 spores/ml).

Among the treatments of liquid formulations of *M. anisopliae* by leaf dip method, *M. anisopliae* @1×10^{12} spores/ml found to better in resulting larval, pupal and adult mortalities of *Spodoptera litura*. In a similar work, Sowmya *et al.* (2017) liquid suspensions of *M. anisopliae* isolate SSB resulted in higher larval mortality, pupal mortality, lowest pupa formation, lowest adult emergence, higher adult mortality at higher concentration under laboratory conditions. Jun (2008) reported the effect of *M. anisopliae* and *N. rileyi* on diamond back moth, *Plutella Xylostella* by leaf dip method and concluded that spore concentration of 10^8 spores/ml resulted in maximum mortalities of various biological stages of *P. xylostella*.In a partially similar work, Khanal *et al.*(2020) [11] reported higher mortality of english grain aphid *Sitobion avenae* under laboratory conditions by leaf dip method of liquid formulations when compared with spray method of liquid formulations.

Among the treatments of wettable powder formulations of *M. anisopliae* by spray method, *M. anisopliae* @1×10^{12} spores/ml resulted in better larval, pupal and adult mortalities of *Spodoptera litura*. In a similar work, Batta (2005) stated higher mortalities of adult stages of *Tribolium castaneum* caused by *M. anisopliae* powder formulation. In a completely contradicted work, Sajid *et al.* (2017) concluded that wettable powder formulations of *M. anisopliae* @1.04 ×10^8 – 5.39 ×10^8 resulted in higher mortality of mustard aphid *Lipaphis erysimi* upto 92%. Our results are coincided with results obtained by Mantzoukas *et al.* (2019) [12] who reported that wettable powder formulations of *M. anisopliae* resulted in larval mortality (43.30%), pupa formation (54.17%) and lower adult emergence (29.14%) in tomato leaf miner *Tuta absoluta*@ 1×10^8 spores/ml.

Among the different treatments of wettable powder formulations of *M. anisopliae* by leaf dip method, *M. anisopliae* @ 1×10^{12} spores/ml resulted in better mortalities of larval, pupal and adult mortalities of *Spodoptera litura*. In a completely contradicted work, Sajid and Khuram Zia (2017) reported that powder formulation of *M. anisopliae* at five different concentrations (5%, 10%, 15%, 20%, 25%) by leaf dip method resulted in highest mortality of 83% in mustard aphid *Lipaphis erysimi*.

Under laboratory conditions, liquid formulations of *M. anisopliae* @1×10^{12} spores/ml by spray method resulted in better larval, pupal and adult mortalities of *S. litura* when compared with liquid formulations by leaf dip method and wettable powder formulations by spray and leaf dip method. *M.anisopliae* results in mycosis of *Spodoptera litura* with 15 days after treatment. Symptoms include development of characteristic green colour sporulation on integument and presence of mycelium werenoticed. These observations were similar to the observations obtained by evaluation of *M. anisopliae* against tomato leaf miner *Tuta absoluta* by Contreras *et al.* (2014) [4].

Conclusion

The present study revealed that liquid formulations of *M. anisopliae* @ 1×10^{12} spores/ml by spray method is better in causing larval, pupal and adult mortalities (52.5%,35%and 22.5%) when compared with liquid formulations by leaf dip method and wettable powder formulations by spray and leaf dip method. Effectiveness of *M. anisopliae* increases against biology of *Spodoptera litura* with increase in spore concentration.

References

1. Ahmad M, Sayyed AH, Saleem MA, Ahmad M. Evidence for field evolved resistance to newer insecticides in Spodoptera litura (Lepidoptera: Noctuidae) from Pakistan. Crop Protection,2008:27(10):1367-1372.
2. Asi MR, Bashir MH, Afzal M, Zia K, Akram M. Potential of entomopathogenic fungi for biocontrol of Spodoptera litura Fabricius (Lepidoptera: Noctuidae). The Journal of Animal and Plant Sciences,2013:23(3):913-918.
3. Batta YA, Abu Safieh. D.I. A study of treatment effect with Metarhizium anisopliae and four types of dusts on wheat grain infestation with red flour beetle (Tribolium castaneum Herbs, Coleoptera: Tenebrionidae). IUG Journal of Natural Studies,2005:13(1):11-22.
4. Contreras J, Mendoza JE, Martinez-Aguirre MR, Garcia-Vidal L, Izquierdo J, Bielza P. Efficacy of enthomopathogenic fungus Metarhizium anisopliae against Tuta absoluta (Lepidoptera: Gelechiidae). Journal of Economic Entomology,2014:107(1):121-124.
5. Dayakar S, Kanaujia KR. Evaluation of the pathogenicity of Beauveriabassiana, Metarhizium anisopliae and Nomuraea rileyi on different larval stages of Tobacco caterpillar, Spodoptera litura (F.). Indian Journal of Plant Protection,2003:31(2):9-12.
6. Halawa B, Azwana A, Panggabean EL. Sensitivity of Larva Spodoptera litura Against the Density of Spores of Fungi Metarhizium anisopliae on the Onion Plant Red (Allium cepa) in the Laboratory. Budapest International Research in Exact Sciences (BirEx) Journal,2019:1(1):35-41.
7. Hussain A, Ahmed S, Shahid M. Laboratory and field evaluation of Metarhizium anisopliae var. anisopliae for controlling subterranean termites. Neotropical entomology,2011:40(2):244-250.
8. Indriyanti DR, Putri RIP, Widiyaningrum P, Herlina L. Density, viability conidia and symptoms of Metarhizium anisopliae infection on Oryctes rhinoceros larvae. Journal of Physics: Conference Series, 2017, 824(012058).
9. Jun MA. Laboratory susceptibility of Plutella xylostella to Metarhizium anisopliae and Nomuraea rileyi. Insect Science,2008:7(1):53-57.
10. Karthikeyan A. Studies on enhancing the efficacy of certain Entomopathogenic fungi against key insect pests of cotton. Ph.D. Thesis, Annamalai University, Chidambaram, 2012, 229.
11. Khanal D, Maharjan S, Lamichhane J, Neupane P, Sharma S, Pandey P. Efficacy of Biorational Compounds against Mustard Aphid (Lipaphis erysimiKalt.) and English Grain Aphid (Sitobion avenae Fab.) under Laboratory Conditions in Nepal. Advances in Agriculture,2020:2020:7.
12. Mantzoukas S, Denaxa NK, Lagogiannis I. Efficacy of Metarhizium anisopliae and Bacillus thuringiensis against tomato leafminer Tuta absoluta Meyrick (Lepidoptera: Gelechiidae). Current Agricultural Research Journal,2019:7(1):37
13. Nahar P, Ghormade V, Deshpande MV. The extracellular constitutive production of chitin deacetylase in Metarhizium anisopliae: possible edge to entomopathogenic fungi in the biological control of insect pests. Journal of invertebrate pathology,2004:85(2):80-88.
14. Panse VG, Sukhatme PV. Statistical methods for Agricultural workers. ICAR, New Delhi, 1978, 238.
15. Romero-Arenas O, Rivera A, Aragon A, Parraguirre C, Cabrera E, Lopez F. Mortality evaluation of armyworm (Spodoptera frugiperda JE Smith) by using Metarhizium anisopliae *in vitro*. Journal of Pure & Applied Microbiology,2014:8(2):59-67.
16. Sajid M, Zia K. *In vitro* efficacy of biopesticide (Beauveria bassiana, Metarhizium anisopliae, Bacillus thuringiensis) against mustard aphid Lipaphis erysimi Kalt. (Hemiptera: Aphididae). Plant Protection,2017:1(2):85-90.
17. Sajid M, Bashir NH, Batool Q, Munir I, Bilal M, Jamal MA, *et al*. In-vitro evaluation of biopesticides (Beauveria bassiana, Metarhizium anisopliae, Bacillus thuringiensis) against mustard aphid Lipaphis erysimi kalt. (Hemiptera: Aphididae). Journal of Entomology and Zoology Studies,2017:5(6):331-335.
18. Sowmya G, Ramanagouda SH, Jayappa J. Lethal and sub lethal effects of indigenous isolates of Metarhizium anisopliae (Metchnikoff) Sorokin against Spodoptera litura (Fabricius). Journalof Entomology and Zoological studies,2017:5(4):1154-1160.