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Evaluation of Bio-agents on Egg Hatching and Larval Mortality of Root-Knot Nematode (*Meloidogyne incognita*)

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Abstract **– In vitro experiments was conducted to study the efficacy of different bio-agents Viz., *Trichoderma viride*, *Paecilomyces lilacinus*, *Pseudomonas fluorescens* and *Pasteuria penetrans* on egg-hatching and larval mortality of M. incognita. All the bio-agents suppressed egg hatching and viability of freshly hatched second stage juveniles of the nematode. After 24, 48 and 72 hours of inoculation at all three concentrations, *P. fluorescens* and *P. lilacinus* were on par with each other and were superior over *T. viride* and *P. penetrans* on egg hatching of the Nematode. However, *T. viride* was superior over *P. penetrans* as against untreated check. While, regarding larval mortality of the nematode at 1.0 and 1.5 per cent concentrations and after 24 and 48 hours of inoculation, *P. lilacinus* and *P. penetrans* were on par with each other and were superior over *T. viride*. Whereas *P. fluorescens* was superior over *T. viride*. But at 2 per cent concentration *P. fluorescens* was significantly superior over *T. viride* and was on par with *P. lilacinus* and *P. penetrants*. However, positive correlation was evinced between levels of concentrations, exposure period and the egg hatching and larval mortality.**

Keywords **– Egg-Hatching, Larval Mortality, M. Incognita, *T. Viride. P. Lilacinus, P. Fluorescens, P. Penetrans*.**

I. INTRODUCTION

Root-knot nematode, *M. incognita* is a serious pest on vegetables and is a major limiting factor especially in the commercial production of tomato in many parts of the world, including Afghanistan. The chemical approach for management of this nematode has become less attractive due to increasing environmental concerns. Therefore, as an eco-friendly approach, use of bio-agents for the management of plant pathogens has received much attention during recent years.

Different bio-agents have been tested by various methods including field application, greenhouse application and lab testing throughout the world and different results have been achieved. But, their comparative efficacy on different stages of the nematodes like eggs, eggs hatching and larval mortality have been less studied.

Different concentration of the culture filtrate of four bio-agents Viz., *Trichoderma viride*, *T. harzianum*, *Trichoderma* sp. and *Fusarium* sp. Were tested by Devi & Bora, 2018 in vitro against egg hatching and larval mortality of *Meloidogyne* incognita. In the study, the highest percentage of inhibition of egg hatching and juvenile mortality was reported by *Trichoderma harzianum* followed by *Trichoderma viride* and *Trichoderma* sp.

Indigenous isolates of *Trichoderma* sp. (*T. harzianum* and *T. viride*) were tested by Singh at al. in 2015 on egg hatching and larval mortality of *Meloidogyne incognita*. The result of their studies revealed that the anti-nematodal compound produced in culture filtrates of both the species directly affected the hatching of the nematode eggs and increased larval mortality.

Guru Prasad and Ravinchandra (2018) carried investigations in vitro to evaluate indigenous isolates of bio -

agents Viz., *Trichoderma harzianum, T. viride, Pachonia lecani* with 2×106 cfu/g, *Pseudomonas fluorescens* and *Bacilus subtilis* with 1×108 cfu/g on inhibition of egg hatching and larval mortality. Findings of their study revealed that among indigenous bio-agents tested, maximum inhibition of egg hatching was recorded in *T. harzianum* (23.33%) amounting to 64.82 per cent and larval mortality of 69.00% after 72 hours of incubation.

Rompalli at al. in 2016 evaluated different concentration of the *Trichoderma viride*, *Pseudomonas fluorescens* and *Purpureocillium lilacinum* isolates on egg hatching and mortality of *Meloidogyne* incognita. Based on their study, among the tested bio-agents, about 92% of larval mortality and 90% of egg hatching inhibition were recorded for *T. viride* and *P. fluorescens*.

Indigenous isolates of bio-agents *viz., Trichoderma. viride, Pseudomonas fluorescens, Trichoderma harzianum, Pachonia chlamydosporium, Fusarium oxysporum* and *Aspergillus niger* were tested in vitro on *M. incognita* egg hatching and larval mortality by Narasimha murthy in 2011. Among indigenous bio-agents tested, maximum inhibition of egg hatching (61-68.74 per cent) and larval mortality (30-75.33 per cent) was reported for *T. viride.*

Anjum and Reddy (2013) studied the efficacy of *Trichoderma viride, T. harzianum, Aspergillus niger* (each at 2 × 106 cfu/ml), *Bacillus subtilis* and *Pseudomonas fluorescens* (each as 1 × 108 cfu/ml), along with carbofuran 3G as chemical control and untreated control (distilled water), on egg hatching and larval mortality of *Meloidogyne incognita* under in vitro conditions. Based on the study findings, *T. viride* and *B. subtilis* were the most effective on inhibiting egg hatching while, *T. viride* and *T. harzianum* were the most effective on causing larval mortality.

In support of the above studies and to further compare the efficacy of the major bio-agents, the present investigations were carried out to compare efficacy of two fungal bio-agents (*Trichoderma viride, Paecilomyces lilacinus*) with two bacterial bio-agents (*Pseudomonas fluorescens* and *Pasteuria penetrans*) on egg hatching and larval mortality of *M. incognita* under *in vitro* conditions. Based on the reviewed literature it is assumed that the efficacy of the fungal bio-agents are superior compared to bacterial bio-agents, thus based on this hypothesis the study was conducted.

II. MATERIAL AND METHODS

Tomato plants infected by root- knot nematode (*M. incognita*) were collected and the species confirmation was done based on the perennial pattern described by Chitwod (1949). After confirming the species as *M. incognita*, the root portion of the nematode infected tomato plants was cut and washed gently under running tap water. Egg masses were hand picked and surface sterilized with 0.1 per cent sodium hypochlorite (NaOCl) for ten seconds followed by five washings with sterile water. The surface sterilized egg masses were kept for hatching in water in Petri plates.

Commercial formulations of the bio-agents *Viz., T. viride* (with 2×10^6 cfu/g), *P. lilacinus* (with 1×10^9 cfu/ml) and *P. fluorescens* (with 1×10^8 cfu/g) were purchased from market and spore suspension of *P. penetrans* (1.5×10^5 cfu /ml) was prepared in Nematology laboratory by the method as described by Walia and Mehta (2001). Carbofuran 3G was used as chemical check and distilled water was used as untreated check. Three different concentrations *Viz.,* 0.5, 1.0 and 1.5 per cent for egg hatching and 1.50, 1.5 and 2.0 per cent of each commercial formulation were prepared for larval mortality using distilled water. Ten ml of each dilution was po-

-ured into sterilized Petri plates and each treatment was replicated three times.

The nematode infected roots from stock cultures were washed under gentle stream of water to remove adhering soil particles. Uniformly sized egg masses were carefully hand picked from the galls with the help of forceps. The collected egg masses were transferred to a sterile beaker and surface sterilized with 0.1 per cent sodium hypochlorite (NaOCl) for 10 seconds followed by five washings with sterile water. Five equal sized fresh egg masses of *M. incognita* with an average of 216 eggs/egg-mass were placed in 10 ml suspension of the bio-agents in different dilutions contained in sterilized Petri plates and incubated at room temperature. The Petri plates having only sterilized water served as control. Each treatment was replicated three times. The plates were examined after 24, 48, 72 hours for hatching of juveniles and the number of juveniles hatched was counted at each interval.

The freshly hatched juveniles (J_2) of *M. incognita* used in this experiment were obtained from egg masses of the nematode maintained on tomato roots. One hundred second stage juveniles of the *M. incognita* were placed in 10 ml suspension of bio-agents in different concentrations (as mentioned in previous studies) separately contained in sterilized Petri plates and was incubated at room temperature. The plates were examined after 24, 48, 72 hours and the number of dead juveniles in each concentration were counted. For each concentration, three replicates were maintained. The juveniles inoculated in carbofuran 3G served as chemical check and the juveniles inoculated in sterile distilled water were taken as untreated check.

III. Result and Discussions

Egg Hatching

The effect of three different concentrations (0.5, 1.0 and 1.5%) of the bio-agents was tested for their ability to inhibit egg hatching of M. incognita. The data recorded on inhibition of egg hatching by bio-agents twenty four hours after inoculation (24 HAI), are presented in Table 1. Minimum egg hatching was observed in carbofuran 3G (8.83%) amounting to 42.29 per cent inhibition over untreated check and the maximum were recorded in untreated check (15.30%). However, the minimum egg hatching among the bio-agents was observed at 0.5 per cent concentration in case of *P. fluorescens* (10.00%) amounting to 34.64 per cent followed by *P. lilacinus* (10.20%) amounting to 33.34 per cent, *T. viride* (11.85%) amounting to 22.54 per cent and *P. penetrans* (11.92%) amounting to 22.09 per cent inhibition over untreated check respectively.

Table 1. Comparative efficacy of bio-agents on egg hatching of *M. incognita* 24 hours after inoculation.

Treatments	Egg hatching (%)					
	Concentration of commercial formulations (%)					
	0.5	Inhibition over control (%)	1.0	Inhibition over control (%)	1.5	Inhibition over control (%)
T_1- *Trichoderma viride*	11.85	22.54	11.00	28.11	10.04	34.38
T_2- *Paecilomyces lilacinus*	10.20	33.34	09.33	39.02	09.33	39.02
T_3- *Pasteuria penetrans*	11.92	22.09	10.50	31.37	10.86	29.02
T_4- *Pseudomonas fluorescens*	10.00	34.64	09.25	39.54	09.78	36.08
T_5- Chemical check (carbofuran 3G)	08.83	42.29	08.25	46.08	07.84	48.76

Treatments	Egg hatching (%)					
	Concentration of commercial formulations (%)					
	0.5	Inhibition over control (%)	1.0	Inhibition over control (%)	1.5	Inhibition over control (%)
T_6- Untreated check	15.30	-	15.30	-	15.30	-
SEm ±	0.82	-	0.98	-	2.15	-
CD at 1%	2.54	-	3.00	-	6.62	-

Similarly at 1.0 per cent concentration of the bio-agents the minimum egg hatching was recorded in carbofuran 3G (5.25%) amounting to 46.08 per cent inhibition over untreated check (15.30%). There was no significant difference among the bio-agents in respect to the inhibition of egg hatching at 1.0 per cent concentration. At 1.5 per cent concentration, minimum egg hatching among the bio-agents was observed in case of *P. lilacinus* (9.33%) amounting to 39.02 per cent followed by *P. fluorescens* (9.78%) amounting to 36.08 per cent, *T. viride* (10.50%) amounting to 34.38 per cent and *P. penetrans* (11.00%) amounting to 29.02 per cent inhibition of egg hatching over untreated check respectively. In general, at all three concentrations and 24 hours after of inoculation, *P. fluorescens* and *P. lilacinus* were on par with each other and were superior over *T. viride* and *P. penetrans*. *T. viride* was superior over *P. penetrans* as against untreated check.

Forty eight hours after inoculation at 0.5 per cent concentration minimum egg hatching was observed in carbofuran 3G (10.80%) amounting to 58.09 per cent inhibition of egg hatching over untreated check (25.77%). All the bio-agents had significant effect on egg hatching compared to untreated check. The minimum egg hatching among the bio-agents was observed in case of *P. lilacinus* (23.58%) amounting to 8.49 per cent followed by *P. fluorescens* (23.97%) amounting to 6.98 per cent and *T. viride* (25.52%) amounting to 0.97 per cent inhibition of egg hatching over untreated check respectively (Table 2). Similarly at 1.0 per cent concentration, the minimum egg hatching was recorded in carbofuran 3G (10.50%) amounting to 59.25 per cent inhibition of egg hatching over untreated check.

Table 2. Comparative efficacy of bio-agents on egg hatching of *M. incognita* 48 hours after inoculation.

Treatments	Egg hatching (%)					
	Concentration of commercial formulations (%)					
	0.5	Inhibition over control (%)	1.0	Inhibition over control (%)	1.5	Inhibition over control (%)
T_1- *Trichoderma viride*	25.52	00.97	23.40	09.17	21.45	16.79
T_2- *Paecilomyces lilacinus*	23.58	08.49	21.17	17.85	18.55	28.02
T_3- *Pasteuria penetrans*	26.42	-2.52	25.48	01.13	22.92	11.06
T_4- *Pseudomonas fluorescens*	23.97	06.98	24.00	06.87	20.36	21.00
T_5- Chemical check (carbofuran 3G)	10.80	58.09	10.50	59.25	08.07	68.68
T_6- Untreated check	25.77	-	25.77	-	25.77	-
SEm ±	1.32	-	1.88	-	1.95	-
CD at 1%	4.08	-	5.78	-	6.00	-

Minimum egg hatching among the bio-agents was observed in case of *P. lilacinus* (21.17%) amounting to 17.85 per cent followed by *T. viride* (23.40%) amounting to 9.17 per cent, *P. fluorescens* (24.00%) amounting to 6.87 per cent and *P. penetrans* (25.48%) amounting to 1.13 per cent inhibition of egg hatching over untreated check respectively. Similar trend was observed at 1.5 per cent concentration, the minimum egg hatching among the bio-agents was observed in case of *P. lilacinus* (18.55%) amounting to 28.02 per cent followed by *P. fluorescens* (20.36%) amounting to 21.00 per cent, *T. viride* (21.45%) amounting to 16.79 per cent and *P. penetrans* (22.92%) amounting to 11.06 per cent inhibition of egg hatching over untreated check respectively. In general, at all the three concentrations and 48 hours after inoculation *P. fluorescens* and *P. lilacinus* were found to be on par with each other and were superior over *T. viride* and *P. penetrans*. And *T. viride* was superior over *P. penetrans* as against untreated control.

Seventy two hours after inoculation, at 0.5 per cent concentration the minimum egg hatching was observed in carbofuran 3G (11.75%) amounting to 76.85 per cent inhibition of egg hatching over untreated check (48.89%). At 0.5 per cent concentration, there was a significant difference among the bio-agents regarding their effect on egg hatching. Minimum egg hatching was observed in case of *P. lilacinus* (36.24%) amounting to 25.87 per cent followed by *P. fluorescens* (38.30%) amounting to 21.66 per cent, *P. penetrans* (40.88%) amounting to 16.38 per cent and *T. viride* (41.17%) amounting to 15.79 per cent inhibition of egg hatching over untreated check respectively (Table 3). Similarly at 1.0 per cent concentration there were significant differences among the bio-agents.

Table 3. Comparative efficacy of bio-agents on egg hatching of *M. incognita* 72 hours after inoculation.

Treatments	**Egg hatching (%)**					
	Concentration of commercial formulations (%)					
	0.5	**Inhibition over control (%)**	**1.0**	**Inhibition over control (%)**	**1.5**	**Inhibition over control (%)**
T_1- *Trichoderma viride*	41.17	15.79	40.50	17.16	38.00	22.27
T_2- *Paecilomyces lilacinus*	36.24	25.87	34.00	30.46	31.83	34.90
T_3- *Pasteuria penetrans*	40.88	16.38	39.33	19.55	38.62	21.00
T_4- *Pseudomonas fluorescens*	38.30	21.66	36.33	25.70	32.80	32.91
T_5- Chemical check (carbofuran 3G)	11.32	76.85	11.75	75.97	08.76	82.08
T_6- Untreated check	48.89	-	48.89	-	48.89	-
SEm ±	0.45	-	0.76	-	1.37	-
CD at 1%	1.38	-	2.34	-	4.23	-

The minimum egg hatching was observed in case of *P. lilacinus* (34.00%) amounting to 30.46 per cent followed by *P. fluorescens* (36.33%) amounting to 25.70 per cent, *P. penetrans* (39.33%) amounting to 19.55 per cent and *T. viride* (40.50%) amounting to 17.16 per cent inhibition of egg hatching over untreated check respectively. Similar trend was observed at 1.5 per cent concentration. Minimum egg hatching was recorded in *P. lilacinus* (31.82%) amounting to 34.90 per cent followed by *P. fluorescens* (32.80%) amounting to 32.91 per cent, *T. viride* (38.00%) amounting to 22.27 per cent and *P. penetrans* (38.62%) amounting to 21.00 per cent

inhibition of egg hatching over untreated check respectively. In general, at all the three concentrations and 72 hours after inoculation among the bio-agents, *P. fluorescens* and *P. lilacinus* were on par with each other and were superior over *T. viride* and *P. penetrans. T. viride* was superior over *P. penetrans* as against untreated check.

The inhibition of egg hatching by fungal and bacterial bio-agents observed here might be due to production of nematicidal compounds (2, 4 -diacetyl phloroglucinol, phenazin-1-carboxylic acid, gliotoxin, viridin, pyrones, peptabiols, tricholin, antibiotics, 6-penthyl-α-pyrone, massoilactone, gliovirin, glisoprenins, heptelidic acid, lilacinin, lewcinostatin, paecilitoxin etc.) by them. Similar reports on inhibition of egg hatching by *Paecilomyces lilacinus*, *Trichoderma viride*, *P. fluorescens* and *P. penetrans* have been reported by Devi and Bora (2018); Guru and Ravichandra (2018); Rompalli et al. (2016); Anjum and Reddy (2013); Narasimhamurthy et al. (2011); and Narasimhamurthy (2010) which are in conformity with present findings.

Larval Mortality

Three different concentration Viz., 1.0, 1.5 and 2.0 per cent of the commercial formulations of the selected fungal and bacterial bio-agents were tested for their efficacy on mortality of M. incognita juvenile (J2) under in vitro conditions. The data recorded after 24, 48 and 72 hours after inoculation on the mortality of M. incognita juvenile is presented in Table 4.

Table 4. Comparative efficacy of bio-agents on mortality of second stage larvae (J2) of *M. incognita*.

Treatments	**Larval mortality (%)**								
	Incubation period (hours)								
	24			**48**			**72**		
	Concentrations of commercial formulations (%)								
	1.00	**1.50**	**2.00**	**1.00**	**1.50**	**2.00**	**1.00**	**1.50**	**2.00**
T_1- *Trichoderma viride*	01.67	05.33	10.33	03.33	09.67	14.00	07.67	11.33	26.53
T_2- *Paecilomyces lilacinus*	03.00	08.33	15.67	05.00	11.00	19.00	09.33	19.00	31.67
T_3- *Pasteuria penetrans*	02.67	08.00	14.33	04.33	10.33	20.67	16.00	18.00	38.33
T_4- *Pseudomonas fluorescens*	02.33	07.33	16.00	03.00	11.67	18.00	09.00	16.00	29.00
T_5- Chemical check (carbofuran 3G)	30.00	54.67	68.33	45.67	85.33	100.00	70.33	92.00	100.00
T_6- Untreated check	01.11	01.11	01.11	02.28	02.28	02.28	02.45	02.45	02.45
SEm ±	0.47	1.59	1.63	1.62	1.60	0.77	1.05	1.82	2.15
CD at 1%	1.45	4.9	5.01	4.98	4.94	2.37	3.25	5.61	6.62

Twenty four hours after inoculation, at 1.0 per cent concentrations all the bio-agents showed significant affect on larval mortality as against control. The maximum larval mortality among the bio-agents was observed in of *P. lilacinus* (3.00%) followed by *P. penetrans* (2.67%), *P. fluorescens* (2.33%) and *T. viride* (1.67%) respectively. Similar trend was observed at 1.5 per cent concentration, the maximum larval mortality was observed in case of *P. lilacinus* (8.33%) followed by *P. penetrans* (8.00%), *P. fluorescens* (7.33%) and *T. viride*

(5.33%) respectively. At 2 per cent concentration, the maximum larval mortality was observed in case of *P. fluorescens* (16.00%) followed by *P. lilacinus* (15.67%), *P. penetrans* (14.33%) and *T. viride* (10.33%) respectively. In general, at 1.0 and 1.5 per cent concentrations and after 24 hours of inoculation, *P. lilacinus* and *P. penetrans* were on par with each other and were superior over *T. viride*. Whereas *P. fluorescens* was superior over *T. viride*. But at 2 per cent concentration *P. fluorescens* was significantly superior over *T. viride* and was on par with *P. lilacinus* and *P. penetrans*.

Forty eight hours after inoculation, at 1.0 per cent concentrations, the maximum larval mortality among the bio-agents was observed in of *P. lilacinus* (5.00%) followed by *P. penetrans* (4.33%), *T. viride* (3.33%) and *P. fluorescens* (3.00%) respectively. Similarly at 1.5 per cent concentrations, maximum larval mortality was observed in case of *P. fluorescens* (11.67%) followed by *P. lilacinus* (11.00%), *P. penetrans* (10.33%) and *T. viride* (9.67%) respectively. At 2 per cent concentration there was significant difference in larval mortality among the inoculated and untreated check.

Similarly the maximum larval mortality was observed in case of *P. penetrans* (20.67%) followed by *P. lilacinus* (19.00%), *P. fluorescens* (18.00%) and *T. viride* (14.00%) respectively. In general, at 1.0 per cent concentration after 48 hours *P. lilacinus* and *P. penetrans* were on par and superior over *P. fluorescens* and *T. viride T. viride* was superior over *P. fluorescens*. At 1.5 per cent concentration among the bio-agents, *P. fluorescens* and *P. lilacinus* were on par with each others and were superior over *P. penetrans* and *T. viride*. Whereas, *P. penetrans* was superior over *T. viride*. But at 2 per cent concentration *P. penetrans* and *P. lilacinus* were on par with each other and were significantly superior over *P. fluorescens* and *T. viride*. However, *P. fluorescens* was significantly superior over *T. viride*.

Seventy two hours after inoculation, at 1.0 per cent concentration, there was a significant difference among the bio-agents regarding their effect on larval mortality. The maximum larval mortality was observed in case of *P. penetrans* (16.00%) which was significantly superior over other bio-agents followed by *P. lilacinus* (9.33%), *P. fluorescens* (9.00%) and *T. viride* (7.67%) respectively. Similarly at 1.5 per cent concentrations the maximum larval mortality was observed in case of *P. lilacinus* (19.00%) followed by *P. penetrans* (18.00%), *P. fluorescens* (16.00%) and *T. viride* (11.33%) respectively. Similar trend was followed at 2.0 per cent concentration and there were significant differences in larval mortality among the inoculated as against untreated control. However, *P. penetrans* was significantly superior over other bio-agents and caused maximum larval mortality (38.33%) followed by *P. lilacinus* (31.67%), *P. fluorescens* (29.00%) and *T. viride* (26.53%) respectively.

In general, 72 hours after inoculation at 1.0 per cent concentration, *P. penetrans* was found significantly superior over other bio-agents. Whereas, *P. fluorescens* and *P. lilacinus* were on par with each other and were superior over T. viride. But at 1.5 per cent concentration *P. penetrans*, *P. lilacinus* and *P. fluorescens* were on par with each other and were significantly superior over *T. viride*. At 2.0 per cent concentrations again *P. penetrans* was significantly superior over other bio-agents. Whereas *P. lilacinus* and *P. fluorescens* were on par with each other and were superior over *T. viride*.

From the above data it may be concluded that, there was a positive correlation coefficient among concentrations of the bio-agents, incubation period and larval mortality. With an increase in concentrations and incubation period there was an increase in larval mortality compare to control (Table 4).

These observations suggest that the inhibitory effect of the bio-agents on hatching and mortality of the nematode larvae might be due to the nematotoxic metabolites released by the antagonistic fungi and bacteria. Although the exact mechanism responsible for larval mortality was not understood and also not reported earlier, the possible mechanism might be by the germination of fungal and bacterial spores which could have parasitized larvae of germination of bacteria on larvae that could have produced metabolites which might have further enhanced the larval mortality.

The nemato-fungal antagonists are known to produce peptidal antibiotics such as lilacinin, lewcinostatin and paecilitoxin (Arai et al., 1973). The acetic acid production was observed in culture filtrates of *P. lilacinus* which affect the movement of nematode (Djian et al., 1991). The present findings are also in confirmity with Guru and Ravichandra (2018); Devi and Bora (2018); Anjum and Reddy (2013) and Narasimhamurthy et al. (2011) who earlier reported larval mortality Meloidogyne incognita by fungal and bacterial antagonists.

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Noor Ahmad Popal is born in Logar, Afghanistan on 25th December, 1977. The author obtained master degree in the field of plant pathology from University of Agricultural Sciences (UAS) Bangalore, India in 2008. He has been working as associate professor in the Department of plant protection, Faculty of Agriculture of Kabul University since 2001. He published two research papers" Comparative Efficacy of bio-agents on the management of Root-Knot Nematode (*Meloidogyne incognita*) under greenhouse conditions" and "comparative efficacy of bio-agents on the management of Root - Knot Nematode (*Meloidogyne incognita*) under field conditions" in English language in Afghanistan Agricultural Research Journal (AARI). Additionally he published 10 review papers in local languages in the Journal of Elm-o-Fan published by Agriculture Faculty of Kabul University.