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Impact of biological and chemical management practices on *Alternaria* leaf blight disease and physiological parameters of Asalio (*Lepidium sativum* L.) crop

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Abstract
Medicinal crops are generally high value crops and are often managed by biological management practices to avoid any pesticide contamination in end product. However, during heavy disease incidence, chemical methods are preferred over biological methods to obtain higher and quality yield. The present study was carried out with objective to compare the impact of both the management practices on disease incidence and physiological parameters of the crop including yield. The biological management practice was conducted in three growing seasons (2014/15, 2015/16 & 2016/17) followed by chemical management practice in next consecutive three years with one overlapping year (2016/17, 2017/18 & 2018/19). It was found that the minimum disease incidence of 39.16 percent was recorded under biological method while 28.25 percent was in chemical method. Similarly, higher seed yield was recorded in chemical method and its best treatment has shown distinct relation with physiological parameters. However, in biological method, lower seed yield was recorded and treatment effects on physiological parameters were not very distinct to one another. The correlation coefficient of percent disease incidence with yield was significantly negative in both the tested management methods. In biological management practice, highly significant positive correlation with transpiration rate and disease incidence was recorded. However, under chemical management practice, yield has significant positive correlation with photosynthetic rate. Although lower yield was recorded in biological method but its impact on disease reduction and on physiological parameters was not very specific that means broad spectrum protection. Hence, minimize the chances of specificity either with pathogen or bioagents residing in plant rhizospere besides excluding the chances of resistance to harmful pathogen.

Keywords: *Alternaria alternata,* biological control, chemical control and physiological parameters

1. Introduction

Garden cress (*Lepidium sativum* Linn) also known as Asalio is native to Egypt and West Asia (Gokavi *et al*., 2004) [9]. It's seeds, leaves and roots posses' medicinal properties due to presence of imidazole, lepidine, semilepidinoside A and B (Maier *et al*., 1998) [13], carotenes, ascorbic acid, linoleic acid, oleic acid, palmitic acid, stearic acid (Duke, 1992) [5], sinapic acid and sinapin (Schultz and Gmelin, 1952) [22]. Asalio being an important medicinal plant with significant pharmacological properties has been observed to be generally affected by many fungal pathogens and *Alternaria alternata* (Fr.) Keissler, one among those, causes severe leaf blight in the northern Indian plains. *A. alternata* causes large economic losses of food and feed every year; as it not only produces melanin but also a large variety of secondary metabolites such as carcinogenic alternariol (Ostry, 2008) [17]. This Host selective toxins (HST) produced by *Alternaria* decide the pathogenicity or virulence (Huang, 2001) [11] of the pathogen. The virulence of the pathogenic strains is positively correlated to their capacity to produce the toxins (Paulitz, and Fernando, 1996) [19] and these toxins are able to produce, symptoms in susceptible plants that have same characteristic of the disease caused by the pathogen (Huang, 2001) [11]. Pathogens reach the host cell either through stomatal pore or by disrupting cuticle layer. Disease condition is always related to deviation in physiological functions of plant as cuticle contains several stomatal pores that regulate diffusion of CO_2 into the leaf for photosynthesis and water vapour out into the atmosphere. Reductions in stomatal conductance after pathogen's attack are closely linked with decreases in photosynthetic processes because the photosynthetic rate is tightly regulated by the stomatal control of CO_2 conductance (Michael *et al*., 2012) [15].

Varietal improvement programme of Asalio for disease resistance is at very primary level. Since, the pathogen survive for long period of time in infected plant parts and also have wide host range. Management of disease through fungicides is the best option but it has not been advocated for decades by several researchers owing to health issues whereas biological methods are not found as effective as chemical methods. Therefore, to save the medicinal plant from getting contaminated by pathogen's toxin, the present investigation was undertaken with an objective to compare the effect of biological and chemical disease management strategies on pathogen's attack apart from assessing deviations in physiological activities of crop.

2. Materials and methods

2.1 Experimental site

Two sets of experiments, each of three years, were conducted at research field of AICRP on Medicinal and Aromatic Plants and Betelvine at Jawaharlal Nehru Krishi Vishwa Vidyalaya (22°49'- 22^0 80'N; 78°21'- 80°58'E), Jabalpur during 2014-15 to 2019-20.The soil of experimental site was sandy loam having 26kg N, 29 kg P, 30 kg K per hectare with organic carbon of 0.60 percent and pH 7.5. One set of experiment comprises of biological management strategy while second of chemical management strategy.

2.2 Experimental design and technique

Asalio seeds for sowing were collected from Herbal Garden, Department of Plant Physiology and; bioagents (*Bacillus subtilis* (PSB), *Azatobacte* sp. & *Trichoderma viride*) from Microbes Research and Production Centre, Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur, Madhya Pradesh. The experiment was laid out in Randomized Block Design with six treatments and four replications. The first set of experiment started during Rabi season of 2014-15 and repeated in 2015-16 & 2016-17, respectively with biological treatments as T1 : FYM@ 10t/ha + PSB@ 6gm/L + spray of 0.15% Azadiractin (Nimbecidine 1500ppm); T2 : *Trichoderma* fortified @10gm/kg of FYM + Azatobacter + spray of 0.15% Azadiractin (Nimbecidine 1500ppm); T3 : *Trichoderma* fortified@10g/kg FYM + PSB @ 6gm/L + spray of 0.15% Azadiractin (Nimbecidine 1500ppm); T4 : FYM@ 10t/ha +PSB@ 6gm/L+ Azatobacter + spray of 0.15% Azadiractin (Nimbecidine 1500ppm); T5 : *Trichoderma* fortified @10g/kg of FYM + PSB@ 6gm/L + Azatobacter + spray of 0.15% Azadiractin (Nimbecidine 1500ppm); T6 : Control (FYM@ 10t/ha). Seeds were sown in plots size of 3.0mx2.0m previously mixed with mentioned treatments at depth of 5cm in furrow having row to row spacing of 40cm. However, the second set of experiment with chemicals started during Rabi season of 2016-17 and repeated in 2017-18 & 2018-19. This experiment comprises of T1: Seed treatment with (Carbendazim 12%+ Mancozeb 63%)-75WP @0.30% plus three foliar sprays (FS) with Mancozeb 75WP @ 0.25% first at initiation of disease followed by 15 days interval; T2: Seed treatment with (Carbendazim 12%+ Mancozeb 63%)-75WP @0.30% plus three foliar sprays(FS) with Ridomil MZ 72 WP @ 0.25% first at initiation of disease followed by 15 days interval; T3: Seed treatment with Metalaxyl 35 SD @ 8g/kg seeds plus three foliar sprays (FS) with Copper oxychloride @ 0.30% first at initiation of disease followed by 15 days interval; T4: Seed treatment with (Carbendazim 12%+ Mancozeb 63%)-75WP @0.30% plus three foliar sprays(FS) with Trifloxystrobin 50WG@ 0.05% first at initiation of disease followed by 15 days interval; T5: Seed treatment with (Carbendazim 12%+ Mancozeb 63%)-75WP @0.30% plus three foliar sprays (FS) with Tebuconazole 25EC @ 0.10% first at initiation of disease followed by 15 days interval; T6: Control. The plot size, sowing time, depth and spacing remained same as followed in first set of experiment except any previous soil incorporations/ treatments. The two experiments were conducted on different piece of land to avoid any overlapping.

2.3. Disease assessment and Physiological parameters

2.3.1 Scaling and calculation of disease incidence

The pathogen from infected leaves was isolated and purified on potato sucrose agar (PSA) medium by hyphal tip method (Ganie *et al.* 2013) and re-infected on randomly (one) selected plant of any plot for confirmation of Koch's postulates. Ten plants per plot sowing typical symptoms were randomly selected, except boarder row plants, were assessed at weekly intervals after first incidence of disease upto 75DAS (days after sowing). Disease assessment was done as per 0–5 scale, based on the percentage of leaf area showing typical symptom, where 0: no visible disease symptoms, 1: < 5% leaf area affected, 2: 5% ≤ leaf area affected < 20%, 3: 20% ≤ leaf area affected < 40%, 4: 40% ≤ leaf area affected < 60%, 5: 60% ≤leaf area affected (Boedo *et al*. (2012) [3]. The Per cent disease index (PDI) was calculated by using the following formula (Wheeler, 1969) [24].

PDI (%) = (∑ [(rating score × number of plants in rating) × 100]/ (total number of sampled plants × highest rating)

2.3.2 Stomatal conductance/ Photosynthetic rate/ Transpiration rate

Stomatal conductance, Photosynthetic rate and Transpiration rate of five leaves/plant of ten plants from each treatment (those selected for disease rating) with four replications have been selected for taking observation and expressed as mean value. The observations were recorded with the help of LICOR-6400(USA make) open type infra red gas analyzer instrument on 75DAS. The mean value of Stomatal conductance (gs), Photosynthetic rate (Pn) and Transpiration rate (E) has been expressed as $molH_2Om^{-2}\ s^{-1}$, $\mu molCo_2\ m^{-2}\ s^{-1}$ and $mmolH_2Om^{-2}\ s^{-1}$, respectively.

2.4 Statistical Analysis

The data recorded under the study were subjected to analysis of variance through randomized block design with help of WASP statistical package of 2.0 versions, Developed and Maintained by AKMU, ICAR-CCARI, Ela, Old Goa. Correlation was also calculated with disease incidence and physiological parameters of crop.

3 Results

3.1Disease incidence and physiological changes under biological management practices

The disease incidence ranged between 33.50percent (T3) to 53.00percent (T6). Treatment effect of T2(37.50%) was second best after T3 while rest were statistically at par with each other and higher than control(T6). Stomatal conductance was maximum (0.48 mol m^{-2} s^{-1}) in T3 while the photosynthetic rate was maximum in T5 followed by T4, T3, T1, T2 & T6, respectively. Moreover, the maximum (2.54 mmolm^{-2} s^{-1}) transpiration rate was recorded in T6 and minimum (1.54 mmolm^{-2} s^{-1}) in T3 while the highest (16.25q/ha) seed yield was recorded in T3 and minimum (12.58q/ha) in T6 during year 2014-15[Fig.1:(a)]. Although during 2015-16, the disease incidence was higher in comparison to previous year but treatment effect of T2 (39.00%) was superior in minimizing the disease incidence to rest as recorded in earlier year of experiment. The highest (0.38 mol m^{-2} s^{-1}) stomatal conductance was recorded in T2 followed by T1. Maximum (20.11 µmolm^{-2} s^{-1}) photosynthetic rate was recorded in T1 followed by T2, T3, T5,T4 &T6 while reverse chronological effect of treatment was observed on transpiration rate with exception to T1 andT2[Fig1:(b)]. The crop yield ranged between 14.49q/ha (T2) to 9.08(T6). Almost identical seed yield was recorded in treatment T5 (10.56q/ha) and T4(10.48q/ha) but was lower than T3(11.41q/ha.). Like previous year (2015-16), treatment effect of T2 (41.00%) was the highest followed by T1 (46.50%) while treatment effect of T3 was identical toT1and T5in suppressing the disease incidence as presented in figure 1:(c). The highest (0.36 mol m^{-2} s^{-1}) stomatal conductance was recorded in T2 while least (0.20 mol m^{-2} s^{-1}) in T6. Similarly, maximum (20.24 µmolm^{-2} s^{-1}) photosynthetic rate was recorded in T2 but minimum (2.13 mmolm^{-2} s^{-1}) transpiration rate in same (T2) treatment. Although, identical (11.08q/ha) seed yield was recorded in treatment T1 andT3 but was lower than T2 (13.41q/ha) and followed by T5 (10.83q/ha), T4 (10.58q/ha) and T6 (9.03q/ha), respectively.

Data of three years (2014-17) of experiment were pooled and analyzed that indicates that treatment T2 has significantly reduced the disease incidence in comparison to others. Maximum stomatal conductance was recorded under treatment T2 which was identical to T3 and T5. Effect of biological treatments on photosynthetic rate was similar but higher than control (T6).Almost similar and higher seed yield was recorded in treatment T2 and T3as presented in table1.

3.2 Disease incidence and physiological changes under chemical management practices

The disease incidence recorded during 2016-17 was minimum (34.25%) in treatment T5 and maximum in T6 (60.25%). Similarly, stomatal conductance was maximum in T5 (0.39 mol m^{-2} s^{-1}) and minimum in T6. Although, the highest (22.30 µmolm^{-2} s^{-1}) photosynthetic rate was also recorded in T5 but least (2.11 mmolm^{-2} s^{-1}) transpiration rate was observed in same treatment. Likewise, maximum (13.90q/ha) seed yield was found in T5 followed by T4 (13.31q/ha), T1 (12.43q/ha), T3 (12.23q/ha), T2 (12.18q/ha) and T6 (9.53q/ha), respectively [figure 1 :(d)]. During the year 2017-18, the disease suppressing ability of treatment T5 (26.00%) and T4 (27.50%) were statistically equal and minimum followed by T1(33.00%). The stomatal conductance was highest (0.41 mol m^{-2} s^{-1}) in T5 followed by T4, T1, T2, T3 and T6. Although the maximum (25.22 µmolm^{-2} s^{-1}) photosynthetic rate was also recorded in T5 but chronology of other treatments effect was different from stomatal conductance. The least (1.90 mmolm^{-2} s^{-1}) transpiration rate was recorded in treatment T5 and maximum (2.75 mmolm^{-2} s^{-1}) in T3. There was significant effect of treatments on seed yield over control (T6). The maximum (13.93q/ha) seed yield was recorded in T5 followed T4, T1, T3, T2, T6 [figure 1:(e)]. In year 2018-19, different treatments significantly reduced the disease over control and maximum(25.25%) disease reduction percent was observed in T5. Almost identical and higher stomatal conductance was found in treatment T5 (0.49 mol m^{-2} s^{-1}) and T1 (0.47 molm^{-2} s^{-1}) than others. The maximum (25.61 µmolm^{-2} s^{-1}) photosynthetic rate and minimum (1.31 mmolm^{-2} s^{-1}) transpiration rate was observed in treatment T5. The highest (18.57q/ha) yield was recorded in T5 while least in T6 (9.71q/ha).

On the basis of three years (2016-19) of data (table 2), it was found that the minimum (28.25%) disease incidence and transpiration rate (1.78mmolm^{-2} s^{-1}) was recorded in T5 while maximum (0.43 mol$m^{-2}$$s^{-1}$) stomatal conductance and photosynthetic rate (24.35µmolm^{-2} s^{-1}) was recorded in the same treatment. Similar and higher seed yield was recorded in T5 and T4. Hence, Seed treatment with Carbendazim 12%+Mancozeb 63%-75WP @0.30% combined with three foliar spray of Tebuconazole 25EC @ 0.10% (T5) was found effective in managing the disease and getting better yield.

3.3 Relation between Disease incidence and physiological parameters

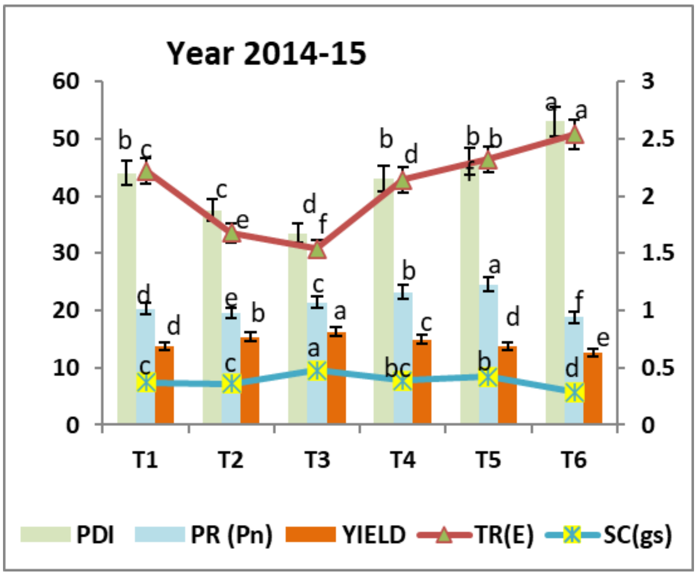
The correlation coefficient of PDI with yield was significantly negative (r= -0.90*& -0.92**) in both the management practices (table3&4). In biological management practice, highly significant positive correlation (r= 0.95** &) with transpiration rate and disease incidence was recorded. Furthermore, there was significant positive correlation of stomatal conductance (r= 0.86*) and highly negative (r= -0.91*) of transpiration was recorded with yield (table3). However, under chemical management practice, yield has significant (r= 0.88*) positive correlation with photosynthetic rate (table 4)

4. Discussion

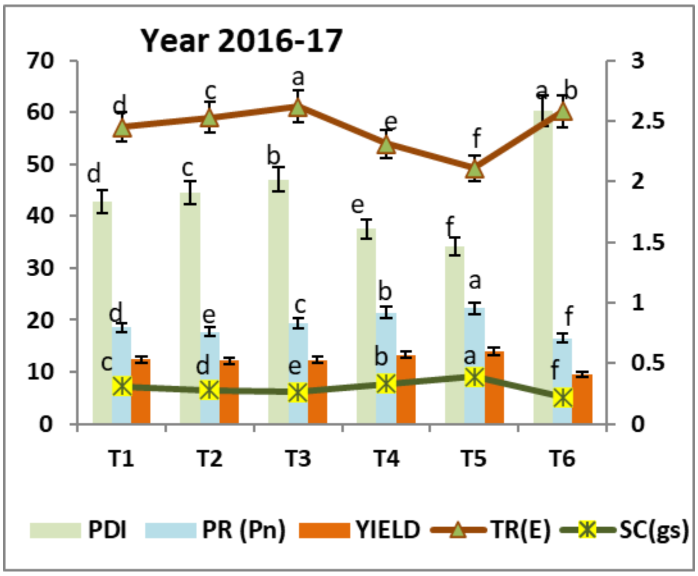
There was significant effect of biological treatments on reducing the disease incidence as bioagents secretes array of antimicrobial and signaling compounds that induces the host resistance mechanisms apart from competing with pathogenic fungi for food and space. Plant rhiosphere is very active and microbial rich and fate of mutual or other association between them depends on nutritional substances released by plant and cross talk of signal molecules received by both for antagonist and competitive interactions (Patel *et al*.,2020) [18]. Therefore, integration of bioagents in disease management tools is found more effective and consistent as it promotes wider crop adaptation. Although, disease incidence was suppressed by different treatments but rate of incidence over the years has increased under biological method. This suggest that the popagules of pathogenic fungi surviving in plant debris or soil have not been killed or managed below damage level by artificially inoculated bioagents. An organism introduced into sterile soil will proliferate and establish itself to a greater extent than introduced into a natural soil because of more intense competition in the more complex ecosystem (Fravel and Keinath, 1991) [6]. Garrett (1956) also reported that it is difficult to introduce successfully an organism into soil of an undisturbed ecosystem. The treatment T3 has successfully managed the disease in first growing season (2014/15) but T2 [*Trichoderma* fortified @10gm/kg of FYM + *Azatobacter* +3 Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm)] out-performed in next two growing seasons (2015/16 & 2016/17). It might be due to synergist association of *Trichoderma viride* with *Azatobacter* sp. where former has induced the resistance mechanism of plant and later made the easy availability of nutrient to its partner. *Trichoderma* species are highly competitive saprophyte of rhizosphere that provide the protection to its host by mycoparasitim, phytoalexin accumulation through induction of host resistance mechanism, antibiotics production and modulation of plant hormones (Harman *et al*., 2004; Yedidia *et al*., 2003; Reino *et al*., 2008 and Martínez-Medina *et al*., 2010) [10, 23, 21, 14] whereas epiphytic and endophytic plant growth promoting bacteria play role in enhancing nutrient efficiency through biofertilization and biostimulation mechanisms (Adesemoye *et al*., 2009) [1]. Under chemical method, there was decrease in disease incidence from Ist growing season (2016/17) to next (2017/18) but again increase was noticed in third growing season (2018/19). Such occurrence of disease could be attributed to the life cycle of infecting pathogen and production of its infecting propagules to overcome the competition with other pathogens. *Alternaria* leaf blight is difficult to manage as *Alternaria* species are polycyclic nature and produces high amount of secondary inoculums that (Campo *et al*., 2007) [4].

Seed treatment with Carbendazim 12%+Mancozeb 63%-75WP @0.30% along-with three sprays with Tebuconazole 25EC @ 0.10% (T5) was found highly effective against the disease because Tebuconazole fungicide inhibit ergosterol biosynthesis.Tebuconazole fungicide inhibits the normal sterol production by demethylation of C-14 during ergosterol biosynthesis that leads to accumulation of C-14 methyl sterols and resulted in slower fungal growth that ultimately lower down its infection potential (Ginoya and Gohel, 2015) [8].

Treatments effect on stomatal conductance was not much significant among them owing to non-specific action of bio-agents under biological management practice. However, significant effect of treatments on transpiration rate and yield was observed as fungal infection led to leaves blightening and; transpiration rate of remaining leaves get increased. Hence, lower photosynthetic rate and higher transpiration resulted in lower yield in heavily infected plants while reverse was recorded in less disease infected plants. It was observed by several workers that the loss of leaves affect the net photosynthesis of the remaining green leaf tissue thereby decreasing the supply of photosynthates to the various sink organs and affecting processes such as grain filling (Lagopodi and Thanassoulopoulos, 1998; Calvet *et al*., 2005) [12, 16]. In chemical method, treatments effect on each physiological parameters *viz*., stomatal conductance, photosynthetic rate, transpiration rate and yield were significant. This could be due to specific and different mode of action of applied fungicides.



A.



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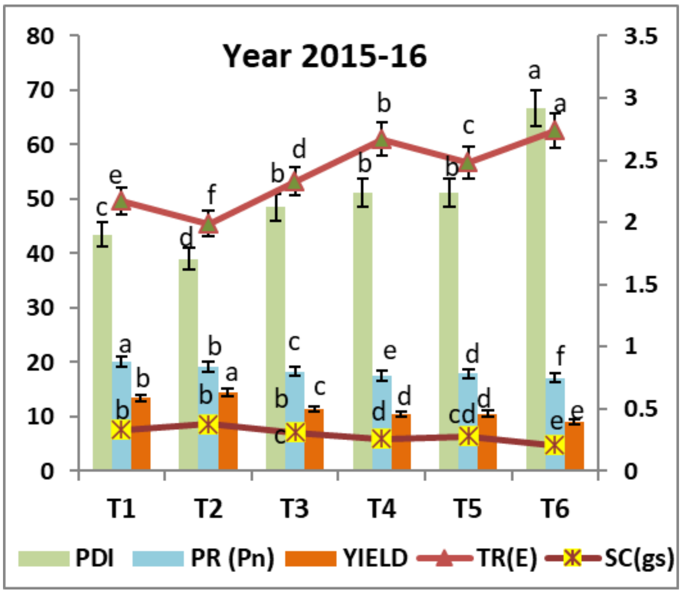

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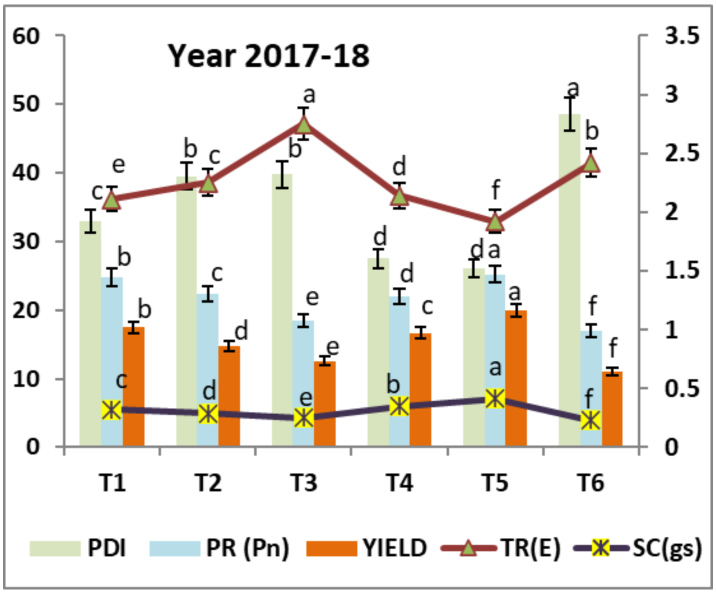

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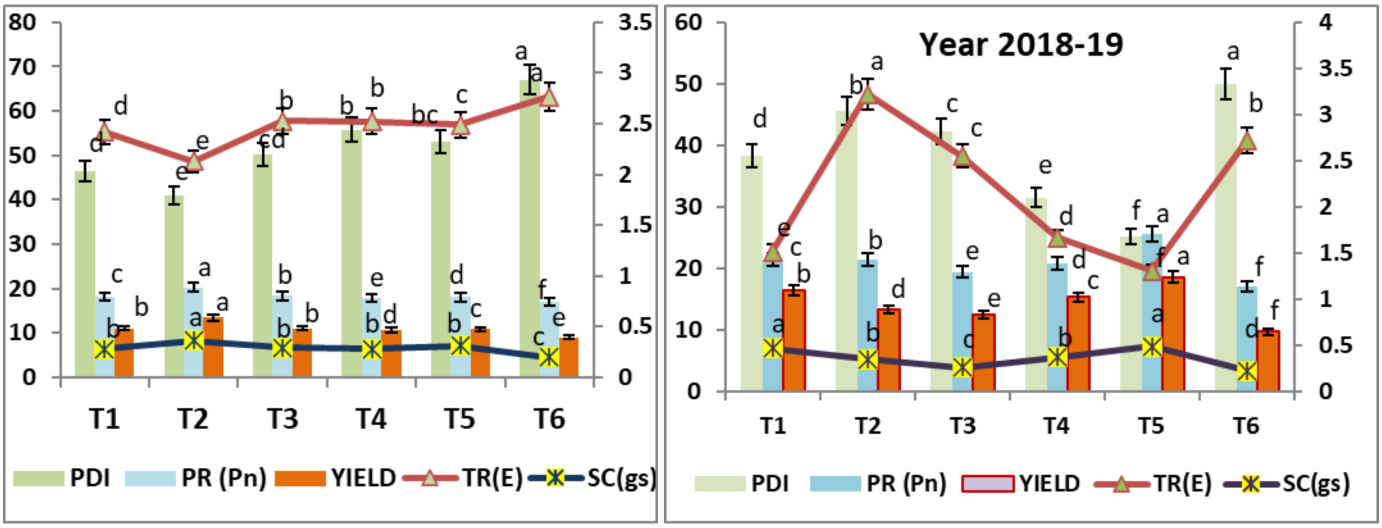

E. F.

Percent Disease Incidence (PDI) has been expressed as percent (%) on Y –Primary axis while Stomatal conductance (gs), Photosynthetic rate (Pn) and Transpiration rate (E) as $molH_2Om^{-2}\ s^{-1}$, $\mu molCo_2\ m^{-2}\ s^{-1}$and $mmolH_2Om^{-2}\ s^{-1}$, respectively on Y – secondary axis. Alphabets (a), (b) & (c) representing biological management practices from year 2014-17while (d),(e)&(f) chemical management practices from year 2016-19

Fig 1: Effect of biological and chemical management practices on disease incidence and physiological components of Asalio crop.

Table 1: Effect of bioagents and bio-formulation on management of *Alternaria* leaf blight of Asalio (*Lepidium sativum*) from year 2014-17

S. No.	Treatment	Percent Disease Incidence (%)	Stomatal Conductance ($mol/m^2/s$)	Photosynthetic Rate ($\mu mol/m^2/s$)	Transpiration Rate ($mmol/m^2/s$)	Yield (q/ha)
1	T1 : FYM@ 10t/ha + PSB@ 6gm/L + Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm)	44.66	0.32	19.52	2.27	12.75
2	T2: *Trichoderma* fortified @10gm/kg of FYM + Azatobacter +3 Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm)	39.16	0.36	19.68	1.93	14.35
3	T3: *Trichoderma* fortified@10g/kg FYM + PSB @ 6gm/L + 3 Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm)	44.08	0.36	19.37	2.13	13.39
4	T4: FYM@ 10t/ha +PSB@ 6gm/L+ Azatobacter + 3Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm)	50.00	0.31	19.55	2.44	12.26
5	T5: *Trichoderma* fortified @10g/kg of FYM + PSB@ 6gm/L + Azatobacter + 3 Foliar spray of 0.15% Azadiractin (Nimbecidine 1500ppm);	50.08	0.33	20.14	2.43	11.74
6	T6: Control (FYM@ 10t/ha).	62.25	0.23	17.67	2.68	11.04
	CD at 5%	3.07	0.03	1.10	0.13	1.00
	CV	7.76	12.45	7.01	6.84	9.73

Table 2: Management of *Alternaria* leaf blight of Asalio (*Lepidium sativum* L) through chemical management practices from year 2016-19

S. No	Treatment	Percent Disease Incidence (%)	Stomatal Conductance ($mol/m^2/s$)	Photosynthetic Rate ($\mu mol/m^2/s$)	Transpiration Rate ($mmol/m^2/s$)	Yield (q/ha)
1	**T1**: Seed treatment with Carbendazim12%+Mancozeb63%-75WP@0.30%+ 3 FS with Mancozeb 75WP @ 0.25%.	38.00	0.36	21.59	2.02	14.56
2	**T2**: Seed treatment with Carbendazim 12%+ Mancozeb 63%-75WP@0.30%+3 FS with Ridomil MZ 72 WP @ 0.25%	43.16	0.30	20.48	2.66	13.42
3	**T3**: Seed treatment with Metalaxyl 35 SD @ 8g/kg seeds + 3 FS with CoC@ 0.30%	43.00	0.25	19.10	2.64	12.58
4	**T4**: Seed treatment Carbendazim 12%+Mancozeb 63%-75WP@0.30%+3FS Trifloxystrobin 50WG@ 0.05%	32.16	0.35	21.39	2.04	15.21
5	**T5**: Seed treatment with Carbendazim 12%+Mancozeb 63%-75WP @0.30% + 3FS with Tebuconazole 25EC @ 0.10%.	28.50	0.43	24.35	1.78	16.74
6	**T6**: Control	52.91	0.22	16.88	2.57	8.52
	CD at 5%	2.04	0.02	1.05	0.25	1.65
	CV	6.29	9.87	6.22	13.73	14.99

Table 3: Effect of different physiological parameters on percent disease index and yield during 2014-17 under biological management practice.

Morphological Traits	Percent Disease Incidence (PDI)	Stomatal Conductance SC(gs)	Photosynthetic Rate PR (Pn)	Transpiration Rate TR(E)	Yield
PDI	1.00				
SC(gs)	-0.73	1.00			
PR (Pn)	-0.27	0.67	1.00		
TR(E)	0.95**	-0.62	-0.26	1.00	
YIELD	-0.90*	0.86*	0.54	-0.91*	1.00

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level

Table 4: Effect of different physiological parameters on percent disease index and yield during 2016-19 under chemical management practice.

Morphological Traits	Percent Disease Incidence (PDI)	Stomatal Conductance SC(gs)	Photosynthetic Rate PR (Pn)	Transpiration Rate TR(E)	Yield
PDI	1.00				
SC(gs)	-0.21	1.00			
PR (Pn)	-0.78	0.22	1.00		
TR(E)	0.70	-0.53	-0.26	1.00	
YIELD	-0.92**	0.49	0.88*	-0.65	1.00

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level

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