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Comparative evaluation of commercial formulations Nutri-Neem and Rifol on downy mildew disease of pearl millet

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ABSTRACT

Pearl millet, *Pennisetum glaucum* (L.) R. Br., is an important dietary food crop with an annual production of 8.01 million tonnes in India. The crop yield is jeopardized by downy mildew pathogen *Sclerospora graminicola*(Sacc.) Schroet causing huge monetary losses. Durable, sustainable ecofriendly approach has become important for management of downy mildew disease in pearl millet in current years. In this study, two commercial SAR formulations i.e., Nutri-Neem (neem oil) and Rifol (combination of fish oil and mineral oil) were evaluated for their growth promoting nature and for their potential to elicit resistance against downy mildew in pearl millet plant. Seed treatment with Nutri-neem recorded maximum seed germination and seedling vigor compared to the Rifol and distilled water control. Under greenhouse conditions 3% Nutri-neem and 7% Rifol as seed treatment recorded 58 and 62 % downy mildew protection respectively which was significantly higher than that of the formulations applied as foliar spray. However, combination of seed treatment and foliar spray showed 75 and 66% downy mildew protection with Rifol and Nutri-neem respectively. Similar downy mildew protection activity was also observed under field conditons for both Rifol and Nutri-neem. The nature of downy mildew protection offered by both Rifol and Nutri-neem was found to be systemic and durable as demonstrated by the spatio-temporal studies. Both Nutri-neem oil and Rifol formulations significantly enhanced the vegetative and reproductive growth parameters of the pearl millet plants compared to the untreated controls. The present study demonstrated that Nutri-neem and Rifol can be applied as growth promoting and resistance inducing formulations for plant disease management in general, and for pearl millet downy mildew in particular.

Key words : Pearl millet, downy mildew, induced systemic resistance, Nutri-neem oil and Rifol formulation.

INTRODUCTION

All plants have the capacity to defend themselves from pathogen attack, either utilizing constitutive defense and/or by the induction of defense responses. The rapidity of inducible responses activated in host for pathogen infection determines the success, failure or disease severity (Dixon *et al*., 1994). Considerable efforts have been made over the past decades in understanding the physiological and biochemical basis of disease resistance in plants. Much of this knowledge is due to the identification of a number of chemical and biological elicitors, some of which are commercially available for use in conventional agriculture. However, the effectiveness of these elicitors to induce disease resistance as a practical means to control various plant diseases is just being realized. The use of various chemicals/abiotic inducers to activate plants resistance using novel alternatives for disease management in many crop systems is well demonstrated (Andreau *et al*., 2006; Taheri and Tarighi, 2010; Wiesel *et al*., 2014). To be considered an activator of SAR, a chemical should have special characteristics. First, the compound or its significant metabolites should not exhibit direct antimicrobial activity; second, it should induce resistance against the similar spectrum of pathogens; and third, it should induce the expression of the marker genes of Systemic Acquired Resistance (SAR) (Kessmann *et al*., 1994). The compounds, such as DL-β-aminobutyric acid (BABA), probenazole, 2,6-dichloroisonicotinic acid and its methyl ester, benzo (l,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), fatty acids, salts of potassium have been shown to induce either SAR genes expression or resistance against pathogens (Walters *et al*., 2013).

Seed treatment with inducers like BABA, calcium chloride, BTH, hydrogen peroxide, plant growth promoting rhizobacteria, bio-agent like cerebrosides, amino acid proline, commercial bioagents formulation Trichoshield, various unsaturated fatty acids have been reported to protect pearl millet plant against downy mildew disease (Shailasree et al., 2001; Geetha and Shetty, 2002; Niranjan Raj et al., 2003; Deepak et al., 2003; Niranjan Raj et al., 2004; Amruthesh et al., 2005).

Recently, several studies have demonstrated the ability of neem extracts in management of many plant diseases, particularly the resistance induction potential of neem products against a broad spectrum of plant pathogens in a wide range of crop plants (Wang, 2010; Goel *et al*., 2016). In this context, the present research was undertaken to evaluate two commercial SAR inducing products Nutri-neem oil and Rifol for their efficacy of induction of resistance in pearl millet against downy mildew disease and also to assess their effect on pearl millet growth parameters.

MATERIALS AND METHODS

Host, Pathogen and Inoculum

Pearl millet seeds cv. 7042S (susceptible to downy mildew) and cv. IP18292 (resistant to downy mildew) were obtained from International Crop Research Institute for Semi Arid Tropics (ICRISAT), Hyderabad, India and All India Co-ordinated Pearl Millet Improvement Project, Mandor, Rajasthan, India and used for the studies. Pathotype 1 of *Sclerospora graminicola* maintained on pearl millet cv. 7042S under greenhouse conditions was used for all the experiments. Leaves of pearl millet showing profuse sporulation of *S. graminicola* on the abaxial side were collected in the evening hours from the plants. The collected leaves were thoroughly washed under running tap water to remove the pre-existing sporangia. The leaves were then blot dried and maintained in a moist chamber for sporulation. The following morning fresh sporangia were washed into distilled water. The resulting zoospore concentration was adjusted to 40,000/ml using a hemocytometer.

Commercial SAR formulations and mode of application

Nutri-Neem a formulation of *Azadirachta indica* seed cold pressed oil 85% was obtained from Nutri-Tech solutions P/L, Queensland, Australia and Rifol is combination of fish oil and mineral oil (Bar Project management, 5 Nitzana St., Israel).

Different concentrations of Nutri-Neem and Rifol was prepared for different treatment modes; 1) Dry seed treatment (0.5, 1, 2, 3, 5, 7, 10 and 15%), 2) Foliar application (diluted using distilled water at the rate of 1:150, 1:200, 1:250) and sprayed on the plants till run off at 3, 7, 14 and 25 day intervals), 3) Dry seed treatment followed by foliar application (a combination of dry seed treatment and foliar spray).

For seed treatment, the seeds of pearl millet were surface sterilized with 0.02% mercuric chloride for 5 min and rinsed thoroughly in distilled water. Seeds

treated with distilled water served as the non-treated control.

Effect of Nutri-Neem oil and Rifol formulation on seed germination and seedling vigor of pearl millet under laboratory conditions

Seed germination test was carried out by paper towel method (ISTA, 1993). Inducer/formulations and distilled water treated seeds were placed onto paper towels. The seeds of pearl millet were placed equidistantly on the presoaked paper. Another presoaked paper towel was placed on the first one so that the seeds were held in position. The paper towels were then rolled and wrapped with polythene to prevent drying. After incubation for 7 days, the towels were unrolled and the number of seeds germinated were counted and percent germination, seedling vigor was analyzed at the end of 7 days according to Abdul Baki and Anderson (1973). The experiment was carried out with four replicates of 100 seeds each and was repeated two times. The length of the root and shoot of individual seedlings were measured to determine the vigor index. The vigor index was calculated using the formula Vigor index = (mean root length + mean shoot length) x (% germination).

Effect of Nutri-Neem oil and Rifol to elicit resistance to downy mildew under greenhouse and field conditions

Seed treatment methods and foliar spray were same as described above for greenhouse and field trials. Seeds treated with distilled water served as the control. Seeds treated with the systemic fungicide Metalaxyl formulation Apron 35 SD at the rate of 6 g per kg served as chemical control. The seeds were sown in clay pots filled with sand, soil and manure at the ratio of 1:2:1. Each treatment consisted of 4 replications, 10 pots per replication and 10 seedlings per pot. Treatments were arranged in a randomized block design. Three day old seedlings were inoculated by the whorl inoculation method (Singh and Gopinath, 1985) with a zoospore suspension of *S. graminicola* at a concentration of 4 x 10^4 zoospores per ml prepared as described earlier. In the whorl inoculation method, droplets of *S. graminicola* zoospores were dropped onto the leaf whorl formed by the emerging seedlings and allowed to flow down to the base. These pathogen inoculated plants were maintained under green house conditions (90 – 95% RH, 20-25 ^{0}C temperature) and observed for disease when they showed any one of the typical downy mildew disease symptoms such as sporulation on the lower surface of leaf, chlorosis, stunted growth or malformation of the earheads. Downy mildew disease incidence was recorded at 30 DAS and final counts were made at 60 DAS.

Field trials were carried out to determine the efficacy of Nutri-neem oil at the pearl millet downy mildew experimental plot. Nutri-Neem oil treatments and the controls were same as previously described. Soilborne oospores of *S. graminicola*, served as the source of primary inoculum. Additional inoculum was provided by infector rows that were raised 21 days prior to the raising of the test rows as described by Williams (1984). Each treatment consisted of four replications. Each replicated row was manually seeded with 100- 150 seeds per row. The experiment was randomized complete block design. Normal agronomic practices were followed to raise the crop. Thinning of excess seedlings were done after 21 days to maintain a uniform number of plants per row and uniform distance between the plants. The crop was irrigated as and when required. The plants were rated diseased when they expressed typical downy mildew symptoms as described above. Downy mildew disease incidence was recorded at 30 DAS and final counts were made at 60 DAS.

Study on resistance induction

Seeds treated with Nutri-Neem oil and distilled water as explained previously were sown to clay pots filled with sand, soil and manure in the ratio of 1:2:1. leaf whorls of 3 day old seedlings were inoculated with a suspension of 4 x 10^4 zoospores per ml of *S. graminicola* as described earlier. A time gap of 1, 2, 3, 4 and 5 days were maintained in different set of plants between the inducer treatment and inoculation with the pathogen. Plants were maintained under greenhouse conditions and were observed for the downy mildew disease reaction. Downy mildew disease incidence data were recorded as described earlier.

Data Analysis

All the experimental results were subjected to Duncan's multiple range test (DMRT). Data on percentages were transformed to arcsine and analysis of variance (ANOVA) was carried out with transformed values. The means were compared for significance using DMRT ($P \leq 0.05$).

RESULTS

Effect of Nutri-Neem oil and Rifol formulation on pearl millet seed germination, seedling vigor, and sporulation of *S. graminicola*

In general, all the evaluated concentrations of the formulations treated to seeds significantly enhanced the seed germination and seedling vigor of pearl millet. However, the enhancement of germination and seedling vigor varied with concentrations and duration of the treatment. Seeds treated for 3, 6, 9 and 12 h duration and in most of the inducers treated seeds at 6 h shown significant germination and seedling vigor was selected for further examination.

All the abiotic inducers tested exhibited improved germination and vigor index at varying degree compared to distilled water control. Optimal concentration was further determined after germination and vigor analysis. Highest percent germination of 95 at different concentrations was recorded in Nutri-neem oil, followed by Rifol, with varying percent germination (92, 91%). However, when compared to distilled water control, all the inducers at different concentration improved the germination. Also maximum seedling vigor (1933) was recorded in Nutri-neem (2 %) treated seeds. Rifol showed maximum seedling vigor recorded (1606) with concentration treated (7 %) seeds. Distilled water control showed vigor index of (1122) (**Table. 1**).

Table 1: Effect of seed treatment with commercial formulations on seed germination, vigour in pearl millet and anti-mildew activity on *Sclerospora graminicola*.

Commercial formulations	Concentration (%)	Germination (%)	Seedling vigor	Sporulation of asexual spores	Zoospore release from sporangia
Nutri-neem oil	2	94 ^{ab}	1933	+++	+++
	3	95 ^{a}	1862	+++	+++
Rifol	5	91 ^{bc}	1526	+++	+++
	7	92 ^{bc}	1606	+++	+++
Distilled water Control	-	84 ^{d}	1122	+++	+++
Apron 35 SD	6gm/kg of seeds	95^{a}	1899	No sporulation	No zoospore

+ = 25 %, ++ = 50%, +++ = 100% sporulation indicates the sporulation and zoospore release from the sporangia. Means followed by the same letter are not significantly different according to DMRT ($P \leq 0.05$).

Table 2: Effect of seed treatment with commercial formulations on downy mildew disease incidence and protection under greenhouse conditions in pearl millet

Commercial formulations	Concentration (%)	Downy mildew disease incidence (%)	Downy mildew disease protection (%)
Nutri-neem oil	1	66 ^{c}	31 ^{e}
	2	44 ^{e}	54^{cd}
	3	40 ^{ef}	58^{c}
Rifol	3	60.5 ^{c}	37 ^{e}
	5	45 ^{e}	52^{cd}
	7	36 ^{f}	62^{b}
Apron 35SD	6g /kg of seeds	9^{h}	91^{a}
Distilled water Control	-	96^{a}	Nil

Results were taken based on four replicates with 100 plants per treatment.

Means followed by the same letter are not significantly different according to DMRT ($P \leq 0.05$).

Effect of seed treatment with Nutri-neem and Rifol on pearl millet downy mildew disease protection

Greenhouse conditions

Seed treatment: Generally, all the concentrations selected for greenhouse studies after germination test performed well in reducing downy mildew disease incidence. However, the levels of disease protection varied with different concentrations tested. The downy mildew disease protection offered due to seed treatment with formulations ranged from 31-62%. The maximum protection was obtained in seeds treated with commercial formulations 3% Neem oil, 7% Rifol protected pearl millet plants 58 and 62 % respectively. However, none of the inducers was par with Apron 35 SD at 6 g/kg of seeds treatment, as it protected pearl millet plants up to 91%. Taken as a whole, compared with distilled water control the performance of the formulations was significant in reducing downy mildew (**Table. 2**).

Foliar spray: In foliar spray treatment of the commercial formulations viz., Nutri-neem oil and Rifol to pearl millet plants protected from 11 – 25% at different concentrations at different day intervals of treatments. The maximum disease protection was observed in Rifol sprayed plants (25%), Followed by Nutri-neem oil (18%) (**Table. 3**).

Seed treatment + Foliar spray: In case of seed treatment followed by foliar spray the trend was changed. The highest disease protection was 75% in 7% Rifol sprayed plants followed by 3% Nutri-neem oil treated plants (66%) compared to distilled water control **(Table. 3**).

Field conditions

Seed treatment: The commercial formulations protected pearl millet plants from downy mildew disease to varying degrees. The promising concentrations under greenhouse studies were selected for field trials. Tested different concentrations performed well in reducing downy mildew disease incidence **(Table. 4)**. The downy mildew disease protection offered due to seed treatment with different concentrations ranged from (58 – 71%). The maximum protection of was obtained in Rifol treated plants (67%) and Nutri-neem (64%), respectively. However, when compared with Apron, formulations treatments were not equal in offering protection, wherein Apron 35SD offered 92% protection against downy mildew disease under field conditions **(Table. 4).**

Table 3: Effect of seed treatment and foliar spray of different commercial formulations on downy mildew disease incidence and protection under greenhouse conditions in pearl millet

Formulations	Mode of treatment	Concentration (%)	Downy mildew disease incidence (%)	Downy mildew disease protection (%)
Nutri-neem oil	Foliar spray	1	84^{ab}	12^{g}
		2	80^{b}	16^{f}
		3	78^{b}	18^{f}
Rifol		3	85^{ab}	11^{g}
		5	80^{b}	16^{fg}
		7	72^{c}	25^{e}
Nutri-neem oil	Seed treatment + Foliar spray	1	49.5^{d}	48^{d}
		2	40.5^{de}	58^{c}
		3	33^{e}	66^{bc}
Rifol		3	35.5^{e}	63^{bc}
		5	30^{ef}	68^{b}
		7	23^{f}	75^{b}
Distilled water Control	Seed treatment	-	96^{a}	Nil
Apron 35SD	Seed treatment	6g /kg of seeds	9^{g}	91^{a}

Results were taken based on four replicates with 100 plants per treatment.

Means followed by the same letter are not significantly different according to DMRT ($P \leq 0.05$).

Table 4: Effect of seed treatment and foliar spray of commercial formulations on pearl millet downy mildew disease incidence and protection under field conditions.

Commercial formulations	Concentration (%)				
Nutri-neem	Seed treatment	2	38^{bc}	58^{c}
		3	32^{c}	64^{bc}
Rifol		5	38^{bc}	58^{c}
		7	30^{c}	67^{b}
Nutri-neem	Seed treatment+ Foliar spray	2	35^{bc}	61^{c}
		3	25.5^{cd}	72^{b}
Rifol		5	31^{c}	65^{bc}
		7	26^{cd}	71^{b}
Apron 35 SD	Seed treatment	6 g/kg of seeds	7^{e}	92^{a}
Distilled water	Seed treatment	-	90^{a}	Nil

Results were taken based on four replicates with 100 plants per treatment.
Means followed by the same letter are not significantly different according to DMRT ($P \leq 0.05$).

Table 5: Effect of seed treatment with commercial formulations on vegetative parameters of pearl millet plants under greenhouse conditions

Abiotic inducers	Height of the plant (cms)	Fresh weight (g) (average per plant)	Dry weight (g) (average per plant)	Number of basal tillers (average per plant)
Nutri neem oil	36.0^{c}	14.20^{b}	5.80^{b}	4.0^{b}
Rifol	40.2^{b}	14.40^{b}	5.80^{b}	4.0^{b}
Distilled water	31.0^{d}	9.95^{c}	4.0^{c}	2.0^{c}

Note: Results were taken 30 days after sowing and are based on the four replicates with 100 plants per treatment.

Table 6: Effect of seed treatment with commercial formulations on reproductive growth parameters of pearl millet under field conditions

Commercial formulations	Height of the plant (cms)	No. of Days required for 50% flowering	Length of earhead/plant	Girth of earhead/ plant	No. of basal tillers/ plant	No. of nodal tillers/ plant	1000 seed weight
Nutri neem	119.2^{b}	41^{b}	11.8^{a}	3.6^{b}	3.5^{b}	3.0^{a}	11.2^{b}
Rifol	131.2^{a}	40^{b}	11.3^{a}	5.2^{a}	4.5^{a}	3.0^{a}	12.8^{a}
Distilled water	112.0^{c}	45^{a}	9.3^{b}	3.1^{b}	2.0^{c}	2.0^{b}	8.6^{d}

Results were taken 60 days after sowing and based on two replicates with 50 plants per treatment.
Means followed by the same letter are not significantly different according to DMRT ($P \leq 0.05$).

Seed treatment + Foliar spray: In seed treatment followed by foliar spray the maximum protection of 72% in Nutri-neem oil treated plants followed by Rifol treated plants at 5 and 7% (65 and 71%) **(Table. 4)**.

Demonstration of induced resistance by time gap studies in pearl millet

After greenhouse studies, 3% Nutri-neem and 7% Rifol was selected for Induction of Systemic Resistance studies. The downy mildew disease protection varied at different day interval inoculation. The maximum protec-

tion obtained in time gap studies of Nutri-neem treated plants protected up to 65 % on fifth day inoculated plants after treatment. The downy mildew disease protection was varied from 52 to 65% at different day intervals of pathogen inoculation. The downy mildew protection was 52, 48, 52 and 62% on 1st, 2nd, 3rd and 4th day inoculated plants respectively (**Fig. 1**).

In case of Rifol treated plants the maximum of 63% downy mildew disease protection was observed on 5th day inoculated after seed treatment. It protected pearl millet plants from 54- 63%. The downy mildew protection was 54, 55 and 64% on 1st, 2nd, 3rd and 4th day inoculated plants respectively over distilled water control (**Fig. 2**).

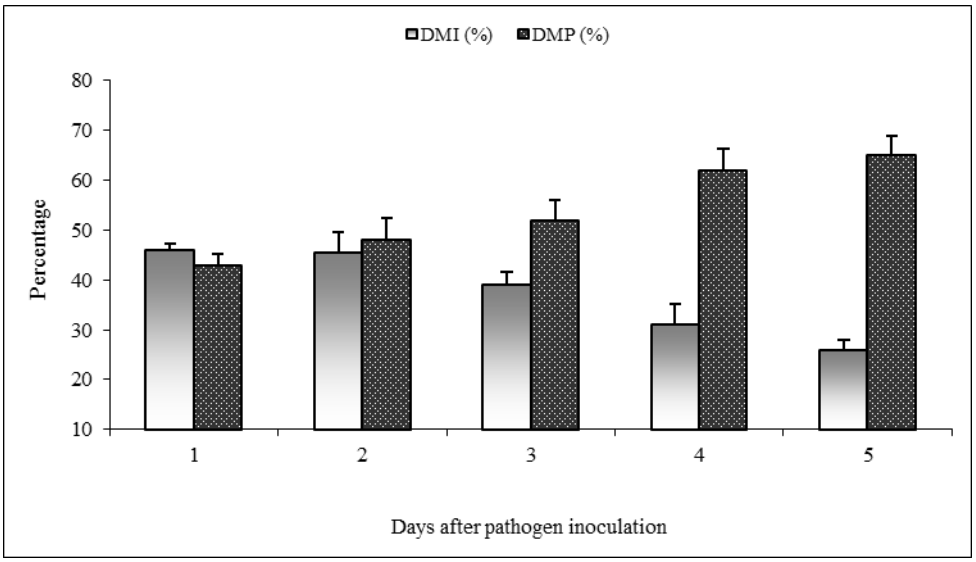

Figure 1: Effect of time gap between Nutri-Neem oil inducer seed treatment and pathogen inoculation on downy mildew disease incidence and protection in pearl millet. Results based on two independent experiments. Vertical bars indicate standard error. DMI – downy mildew incidence, DMP – downy mildew protection.

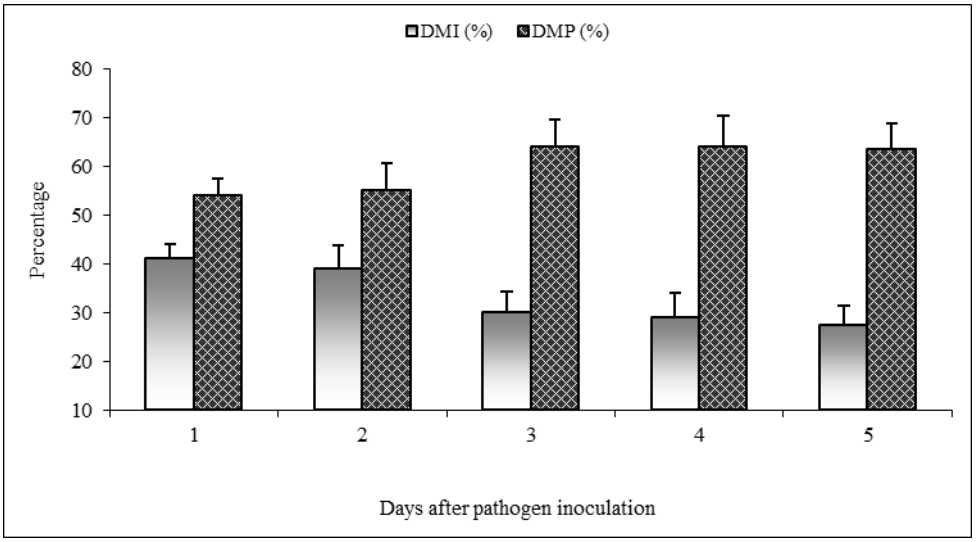

Figure 2: Effect of time gap between Rifol seed treatment and pathogen inoculation with on downy mildew disease incidence and protection in pearl millet. Results based on two independent experiments. Vertical bars indicate standard error. DMI – downy mildew incidence, DMP – downy mildew protection.

Effect of inducers on vegetative and reproductive growth parameters in green house and field

In general, Nutri-neem oil and Rifol seed treatments showed positive growth responses among all the parameters measured under greenhouse conditions compared to non-treated controls. Specifically, the treatments significantly enhanced seedling height compared to control. The height of the pearl millet plants treated with Nutri-neem oil and Rifol was 36 and 40.2 cm whereas in control plants (31cm). Nutri-neem oil and Rifol treatment significantly enhanced shoot fresh weight, which was 14.2 and 14.4 and dry weight of 5.8 and 5.8g respectively. The tillering of the pearl millet plants was also enhanced due to treatment with Nutri-neem oil (4 per plant) and Rifol (4 per plant) in comparison to the control (2.0) **(Table. 5)**

Similarly, the above treatments also significantly enhanced height of the plant, length of ear head, grain yield and reduced days to 50% flowering compared to control under field conditions. Under field conditions the height of the pearl millet plants was 119.2 and 131.2 cm due to Nutri-neem oil and Rifol treatment as against the control (112 cm). Reproductive parameters like the ear head length; girth and grain yield was also enhanced due to inducers treatment. The grain yield (1000 seed weight) was, 11.2 and 12.8 g due to Nutri-neem oil and Rifol treatments over the control (8.6 g). The number of days required for flowering in induced plants was reduced 41 days in Nutri-neem oil and 40 days in Rifol treated pearl millet plants whereas in distilled water control plants required 45 days for flowering. As well as in number of basal and nodal tillers due to treatment with inducers same trend was observed over control (Table. 6).

DISCUSSION

The ability of plants to actively protect themselves from various pathogens/diseases with inducible and constitutive mechanisms has been established in various crop species. These inducible mechanisms can be triggered with appropriate agents termed as elicitors/inducers, which can be either biotic or abiotic in nature and the resistance so developed is termed as systemic acquired resistance (SAR). SAR is projected as an alternative to the use of pesticides for control of plant pathogens. Various chemicals have been discovered that seem to act at various points in these defense activating networks and mimic all or parts of the biological activation of resistance.

In this study, abiotic inducers Nutri-neem oil and Rifol were evaluated for their effectiveness to manage pearl millet downy mildew disease and also to enhance pearl millet growth. The chemicals were evaluated as seed treatment and the formulations were evaluated as seed treatment, foliar spray and combination of seed treatment and foliar spray.

The abiotic inducers tested in the present study at different concentrations did show fungitoxic effect on the fungus *S. graminicola*. There was no inhibition of sporulation and zoospore release, due to the inducer treatment and control, whereas in the metalaxyl treatment there was complete inhibition of fungal sporulation and zoospore release observed.

To ensure that the treatments did not have any negative effect on pearl millet seed germination and seedling vigor, optimization of seed treatment duration and concentration, the abiotic inducers were tested at various concentrations and also for different treatment durations. In general, all the evaluated concentrations of the abiotic inducers treated to seeds significantly enhanced the seed germination and seedling vigor of pearl millet. However, the enhancement of germination and seedling vigor varied with the treatments time and concentrations. Nutri-neem oil and Rifol treatments recorded highest germination of 95%.

There are numerous reports where different chemical inducers have enhanced the seed germination; seedling vigor, plant emergence and plant stand in various crops. Also the same effects have been observed in pearl millet by earlier workers using various inducers like INA, BABA, BTH, $CaCl_2$ and H_2O_2, PGPRs (Shivakumar, 2000; Shailasree *et al*., 2001; Geetha and Shetty, 2002; Niranjan *et al*., 2004; Baysal *et al*., 2005).

Further when these different inducers were tested for their ability to protect pearl millet plants from downy mildew under greenhouse conditions, it was observed that, in general, the inducers offered protection against downy mildew in the range of 31-62%. Nutri-neem, and Rifol were very promising and they offered 58 and 62% protection against pearl millet respectively. However, none of these treatments offered protection equal to that of the Apron treatment, which offered 91 % protection. Our results are in line with many other reports where treatments with different chemicals have offered protection against many diseases in a variety of crops. Particularly treatment of Tri-phosphate, K_2HPO_4,

KH_2PO_4, K_2HPO_3, K_3PO_3, H_3PO_3, Na_2SiO_3, K_2SiO_3, KH_2PO_4, $NaHCO_3$ (Reuveni *et al*.,1997; Pajot *et al*., 2001; Yildirim *et al*., 2002) well demonstrated in powdery mildew of cucumber, downy mildew of lettuce (*Bremia lactucae*), *Vitis vinifera* L, and Gallic acid, Boric acid, copper sulfate and manganese chloride in cucumber powdery mildew caused by *Sphaerotheca fuliginea* (Reuveni *et al*., 1998) and neem products Neemazal in pea (*Pisum sativum)* and Milsana (*Reynoutria sachalinensis)* (Singh and Prithiviraj, 1997; Bowers and Locke, 2004) and turtle oil against bacterial canker in tomato (Baysal *et al*., 2005). Furthermore, various other chemicals like BTH, BABA, INA and Proline was reported to protect pearl millet plant against downy mildew disease when used as seed treatment and other crops (Shivakumar, 2000; Shailasree *et al*., 2001; Geetha and Shetty, 2002; Niranjan *et al*., 2004; Andreau *et al*., 2006).

Similarly, under field conditions Nutri-neem oil and Rifol offered 66 and 75% protection against downy mildew disease. Apron treatment, like in greenhouse conditions, offered higher protection compared to all other treatments and control. None of the treatments in any of these experiments conferred complete protection against downy mildew disease of pearl millet. Even plants treated with metalaxyl did not showed complete protection against downy mildew, which suggests that complete protection is not possible in these experiments due to optimal climatic conditions for infection and high inoculum concentration.

The commercial formulations namely Nutri-neem, and Rifol in addition to seed treatment were also tested as foliar spray and combination of seed treatment and foliar spray. Foliar spray with Nutri-neem, and Rifol offered 18 and 25% protection. But the combination of seed treatment with foliar spray offered 66 and 75% protection, which was significantly higher in comparison to the single treatment alone. This suggests that the inducers might have acted in multiple ways against downy mildew disease. The seed treatment might have induced resistance against the pathogen and the foliar spray might have offered curative action thus enhancing the protection capacity.

There are various other examples where different chemicals have controlled various plant diseases under field conditions (Kim *et al*., 2001). In addition to downy mildew disease reaction, the inducers were also evaluated for their efficiency to promote pearl millet growth. It was found that, the inducers were effective in promoting pearl millet growth both under greenhouse and field conditions. Nutri-neem oil and Rifol seed treatments had positive influences on growth responses among all the parameters measured under greenhouse conditions compared to non-treated controls. Shoot as well as root dry matter increased by 14.2, 14.4g and 5.8g in comparison to the control both under greenhouse and field conditions. Treatments significantly enhanced seedling height, fresh and dry weights, tillering capacity compared to control. Similarly, the above treatments also significantly enhanced the reproductive parameters of pearl millet such as height of the plant, length of ear head, grain yield, and reduced days to 50% flowering compared to control under field conditions. Most importantly the grain yield (1000 seed weight) was 10.8, 11.2 and 12.8% more due to Nutri-neem oil and Rifol treatments over the control. Enhancement of growth using various chemical treatments has been reported earlier in pearl millet and other plants (Shailasree *et al*., 2001; Shivakumar, 2000; Geetha and Shetty, 2002; Niranjan *et al*., 2004).

Seed treatment for 6 h in an aqueous solution of Nutri-neem oil and Rifol reduced the infection of *S. graminicola* and these inducers were applied at 3 and 7% concentration reduced the rate of infection and offered 64 and 67% protection against downy mildew respectively. However, metalaxyl treatment protected 91% of pearl millet plants from downy mildew disease.
The results presented here showed for the first time induction of resistance in pearl millet against downy mildew disease using inducers Nutri-neem oil and Rifol. Our results are in line with earlier reports where potassium salts, sodium salts, copper sulfate, boric acid, gallic acid was reported to stimulate resistance in different plants against a broad spectrum of viral, bacterial, and fungal pathogens (Fought and Kuc, 1996; Reuveni *et al*., 1997; Singh and Prithiviraj, 1997; Reuveni *et al*., 1998; Oostendorp, *et al*., 2001; Pajot *et al*., 2001; Yildirim *et al*., 2002).

Inducers, Nutri-neem and Rifol, which offered highest protection under greenhouse and field conditions, were further evaluated to study the nature of protection offered under greenhouse conditions. The nature of resistance offered was found to be systemic and durable and it took 3-day time gap for the development of resistance. Both spatial as well as temporal separation of the inducing agent and the challenging pathogen, which are the main criteria to induced systemic resistance, were maintained to deduce the nature of protection

offered by, Nutri-neem, and Rifol protected the plants in a systemic way. In addition, inducers tested in the study at different concentrations did not show any fungitoxic effect on the *S. graminicola*, which also indicated that the resistance developed, is of systemic nature. It was also demonstrated that the resistance conferred by these abiotic agents was durable, which was sustained throughout the plant life. Similar results have been reported from other pathosystems (Cohen *et al*., 1993; Jensen *et al*., 1998; Morris *et al*., 1998; Hong *et al*., 1999; Tosi *et al*., 1999; Cohen, 2002; Yusuf and Sally, 2004; Iriti *et al*., 2005). These experiments demonstrated that the use of defense activators Nutri-Neem and Rifol can enhance resistance to downy mildew disease and promote growth of pearl millet plants.

CONCLUSION

The results of the present study suggested that the exogenous application of Nutri-Neem and Rifol as seed treatments to pearl millet plants induce resistance to downy mildew disease. Moreover, the resistance induction is complemented with the growth promotion, which is an added benefit. These treatments were very much effective even when at lower concentrations and single applications, which is very much beneficial for a poor crop like pearl millet. This work suggests the role of systemic acquired resistance in plant disease management and its potential as one of the major inputs in integrated disease management strategy for plant disease management and downy mildew disease management in particular..

REFERENCES

Abdul Baki AA and Anderson JD (1973) Vigour determination in soybean seed by multiple criteria. *Crop Science*, 13 (6): 630 – 633.

Amruthesh KN, Geetha NP, Jørgensen HL., de Neergaard E and Shetty HS (2005) Unsaturated fatty acids from zoospores of *Sclerospora graminicola* induce resistance in pearl millet. *European Journal of Plant Pathology*, 111(2): 125–137.

Andreau AB, Guevera MG, Wolski EA, Daleo GR and Caldiz DO (2006). Enhancement of natural disease resistance in potatoes by chemicals. *Pest Management Science*, 62 (2): 162-170.

Baysal O, Gursoy YZ, Ornek H and Duru A (2005) Enhanced tomato resistance to bacterial canker by application of turtle oil. *Journal of General Plant pathology*, 71(3):204-210.

Bowers JH and Locke JC (2004) Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of *Phytophthora* blight in the greenhouse. *Plant Disease*, 88(1): 11-16.

Cohen Y, Gisi U and Niderman T (1993) Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology*, 83 (10): 1054-1062.

Cohen YR (2002) β-Aminobutyric Acid-induced resistance against plant pathogens. *Plant Disease*, 86 (5): 448-457.

Deepak SA, Niranjan Raj S, Umemura K, Kono T and Shetty HS (2003) Cerebroside as an elicitor for induced resistance against the downy mildew pathogen in pearl millet. *Annals of Applied Biology*, 143 (2): 169-173.

Dixon RA, Harrison MJ and Lamb CJ (1994) Early events in the activation of plant defence response. *Annual Review of Phytopathology,* 32:479–501.

Fought L and Kuc JA (1996) Lack of specificity in plant extracts and chemicals as inducers of systemic resistance in cucumber plants to anthracnose. *Journal of Phytopathology*,144 (1): 1-6.

Geetha HM and Shetty HS (2002) Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide – a comparative evaluation. *Crop Protection*, 21 (8): 601– 610.

Goel N, Anukrati K and Pau PK (2016) Anti-phytopathogenic and SAR inducing properties of neem: a review. *Journal of Chemical and Pharmaceutical Sciences* 9 (4):2547-2555.

Hong JK, Hwang BK and Kim CH (1999) Induction of local and systemic resistance to *Colletotrichum coccodes* in pepper plants by DL-β-Amino-n-Butyric acid (BABA). *Journal of Phytopathology*, 147 (4): 193-198.

Iriti M and Faoro F (2005) Benzothiadiazole (BTH) induces cell-death independent resistance in *Phaseolus vulgaris* against *Uromyces appendiculatus*. *Journal of Phytopathology*, 151 (3): 171-180.

ISTA (1993) Proceedings of the international Seed Testing Association, International Rules for Seed Testing. *Seed Science and Technology,* 21: 25-30.

Jensen BD, Latunde-Dada AO, Hudson D and Lucas JA (1998) Protection of *Brassica* seedlings against downy mildew and damping-off by seed treatment with CGA 245704, an activator of systemic acquired resistance. *Pesticide Science*, 52(1): 63-69.

Kessmann H, Staub T, Hofmann C, Maetzke T and Herzog J (1994) Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology,* 32: 439─459.

Kim YC, Blee KA, Robins J and Anderson AJ (2001) Oxytom TM under field conditions and laboratory conditions increases resistance responses in plants. *European Journal of Plant Pathology*, 107 (1): 129-136.

Morris SW, Vernooij B, Titatarn S, Starrett M, Thomas S, Wiltse CC, Frederiksen RA, Bhandhufalck A, Hulbert S and Uknes S (1998) Induced resistance responses in maize. *Molecular Plant Microbe Interaction*, 11(7): 643-658.

Niranjan Raj S, Deepak SA, Basavaraju P, Shetty HS, Reddy MS and Kloepper JW (2003) Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet. *Crop Protection*, 22(4): 579–588.

Niranjan Raj S, Shetty NP and Shetty HS (2004). Proline - an inducer of resistance against pearl millet downy mildew disease caused by *Sclerospora graminicola. Phytoparasitica*, 32(5): 523-527.

Oostendorp M, Kunz W, Dietrich B and Staub T (2001) Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology*, 107(1): 19─28.

Pajot E, Corre DL and Silue D (2001) Phytogard and DL- *β*-amino butyric acid (BABA) induce resistance to downy mildew (*Bremia lactucae)* in lettuce (*Lactuca sativa* L.). *European Journal of Plant Pathology*, 107: 861-869.

Reuveni M, Agapov V and Reuveni R (1997) A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fuliginia*) in cucumber plants. *European Journal of Plant Pathology*, 103 (7): 581–588.

Reuveni M, Harpaz M and Reuveni R (1998) Integrated control of powdery mildew on field-grown mango trees by foliar sprays of mono-potassium phosphate fertilizer, sterol inhibitor fungicides and the strobilurin Kresoxym-methyl. *European Journal of Plant Pathology*, 104 (9): 853–860.

Shailasree S, Sarosh BR, Vasanthi NS and Shetty HS (2001) Seed treatment with beta-aminobutyric acid protects *Pennisetum glaucum* systemically from *Sclerospora graminicola*. *Pest Management Science*, 57 (8): 721-728.

Shivakumar PD (2000) Induction of systemic resistance in pearl millet against downy mildew disease and the mechanism of resistance. Thesis submitted to University of Mysore, Mysore, India.

Singh SD and Gopinath RA (1985) Seedling inoculation technique for detecting downy mildew resistance in pearl millet. *Plant Disease*, 69 (7): 582– 584.

Singh UP and Prithiviraj B (1997) Neemazal, a product of neem (*Azadiracta indica*), induces resistance in pea (*Pisum sativum)* against *Erysiphe pisi. Physiological Molecular Plant Pathology*, 51 (3): 181-194.

Taheri P and Tarighi S (2010). Riboflavin induces resistance in rice against *Rhizoctonia solani* via jasmonate-mediated priming of phenylpropanoid pathway. *Journal of Plant Physiology,* 167 (3): 201–208.

Tosi L, Luigetti R and Zazzarini A (1999) Benzothiadiazole induces resistance to *Plasmopara helianthi* in sunflower plants. *Journal of Phytopathology*, 147 (6): 365-370.

Walters DR, Ratsep J and Havis ND (2013) Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*, 64 (5):1263–1280.

Wang J, Jian Li, Jiankang Cao and Jiang W (2010) Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. *African Journal of Microbiology Research*, 4 (11): 1100-1104.

Wiesel L, Newton AC, Elliott I, Booty D, Gilroy EM, Birch PRJ *et al*. (2014) Molecular effects of resistance elicitors from biological origin and their potential for crop protection. *Frontiers in Plant Science,* 5:655-667.

Williams RJ (1984) Downy mildew of tropical cereals. In: Advances in plant pathology, vol. 2. D. S. Ingram and P. H. Williams (eds.), Academic Press, London. Pp. 1-103.

Yildirim I, Onogur E and Irshad M (2002) Investigations on the efficacy of some natural chemicals against powdery mildew [*Uncinula necator* (schw.) burr.] of grape. *Journal of Phytopathology*, 150 (11-12): 697-702.

Yusuf Y and Sally W (2004) Effect of treating pepper with Benzothiadiazole derivative and non-pathogenic *Xanthomonas campestris* pathovars on the level of disease caused by *Sclerotinia sclerotiorum*. *Plant Pathology Journal*, 3 (1): 52-55.