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Effect of smoke on insect mortality and quality parameters of stored wheat at Pantnagar, Uttarakhand

Usha Yadav and Ruchira Tiwari

Abstract
A novel study was made on the effect of cow dung and neem leaves derived smoke on mortality of adults of *Sitophilus oryzae*, *Rhyzopertha. dominica*, *Tribolium castaneum*, 3rd and 5th instar larvae and adult moths of *Corcyra. cephalonica* and on quality parameters of stored wheat (*Triticum aestivum* L.) under laboratory conditions in the Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2016. The results showed that more than 50 per cent insect mortality was observed after 72h of exposure to neem leaves smoke and after 96 h of exposure to cow dung smoke. On the other hand, smoke generated by cow dung and neem leaves had no adverse effect on germination of seeds after different exposure time periods. Therefore, the present study clearly revealed that cow dung and neem leave smoke was lethal to storage insect pests but has stimulatory effect on germination and post germination response of seeds.

Keywords: Cow dung, insect pests, neem, quality parameters, smoke, stored wheat

1. Introduction
Wheat (*Triticum aestivum* L.) is the world's most important cereal crop in relation to production and consumption [1]. India is the second largest producer of wheat with a total production of about 96.0 million tonnes and 29.8 million hectare area under cultivation [2].
Wheat is attacked by various insect pests between harvest and storage. The most economically important insect pests of stored wheat are the rice weevils, *Sitophilus oryzae*, lesser grain borer, *Rhizopertha dominica*, rice moth, *Corcyra cephalonica*, Angoumois grain moth, *Sitotroga cerealella*, Indian meal moth, *Plodia interpunctella* and red rust flour beetle, *Tribolium castaneum* [3]. The insects and other pests severely deteorate agricultural stored products and are responsible for worldwide losses of stored grains ranging from 10 to 40 per cent per annum [4].
The control of these insect pests around the world is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticide and fumigants such as methyl bromide and phosphine. Development of insect resistance to phosphine has been also reported [5]. Their uninterrupted and indiscriminate use not only had led to the development of resistant strains but also accumulation of toxic residues on food grains used for human consumption that leads to the health hazards [6]. In this context, to avoid the injudicious use of synthetic chemicals the naturally produced smoke has a great potential for causing insect mortality and promoting seed germination and enhancing plant growth. This easily accessible technology is a good substitute for hazardous chemicals and can easily to adopt by the users without having any adverse effects on stored wheat grains and human health. Keeping all these facts in mind the present study was made to quantify the possible effect of smoke generated by burning of cow dung cake and neem leaves separately on mortality of stored insect pests and on quality of stored wheat seeds under airtight conditions.

2. Materials and Methods
2.1 Collection of materials and Production of Smoke
The seeds of wheat variety UP-2338 was taken from Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2016. The cow dung cakes were collected from desi breed cow from the nearby houses domesticating cows whereas neem leaves were collected from the campus areas. Smoke was produced by burning of dried cow dung cake and semi dried neem leaves, separately in a smoker and aerosol smoke was collected for further process.

The insect culture of test insects *viz*. *S. oryzae, R. dominica, T. casteneum* and *C. cephalonica* were obtained from the stock culture prepared on stored wheat in the laboratory of Department of Entomology, ant university, Uttarakahnd

2.2 Bioassay on effect of smoke on the mortality of test insects

The laboratory bioassasys were conducted on the effect of smoke generated by burning of neem leaves and cow dung cake, separately on the mortality of major stored insect pests such as adult weevils of *S. oryzae*, adult beetles of *R. dominica*, *a*dult moths, 3rd instar and 5th instar larvae of *C. cephalonica* and adult beetles of red rust flour beetle, *Tribolium casteneum* by releasing 50 insects each, separately in conical flasks in three replications (Total number of insects =150) containing 5 g of wheat grains. After releasing insects, smoke was filled in conical flasks by burning of neem leaves and cow dung cake, separately with the help of smoker and opening of the conical flasks were tightly closed with cotton plugs. The experiments were set up separately for different interval of time *i.e.*1, 6, 12, 24, 48, 72, 96 and 120 h to find out the effect of smoke on the all tested insects under smoke exposure for different time periods.

2.3 Effect of smoke on seed quality parameters at different exposure time interval

The effect of smoke generated by cow dung cake and neem leaves on quality of wheat seeds was studied on the parameters of germination and post germination response of treated wheat seeds. The wheat seeds were kept in conical flasks treated with cow dung smoke and neem leaves smoke, separately for different exposure time periods under airtight conditions. The effect of smoke on seed quality was assessed on different quality parameters by using Germination assay [7].

Germination assay was conducted to find out the effect of smoke on the germination and post germination quality parameters. A piece of filter paper was spread in to a plastic boxes of size 8x4x3 cm^3 each and 5 ml of distilled water was added. Treated seeds were then transferred onto the filter paper, in separate plastic boxes and covered with lids containing mesh for aeration and then placed at an ambient temperature. Germination counts were taken on a daily basis for 10 days. After 10 days, root and shoot length were measured. Then, percent seed germination, germination index, vigor index and significance of viability were calculated. Germination index was calculated using formula [8] and seedling vigor index and significance of viability were calculated by using equation [9].

$$\% \text{ Germination} = \frac{\% \text{ Seeds germinated} \times 100}{\text{Total no of seeds planted}}$$

$$\text{Germination Index} = \frac{\text{No. of seedlings emerged on day (n)}}{\text{Days after planting (d)}}$$

Vigor Index (VI) = % germination x (root length + shoot length)

$$\text{Significance of Viability (SV)} = \frac{\% \text{ germination in treated grains}}{\% \text{ germination in control}}$$

2.4 Statistical analysis: Data was subjected to Complete Randomized Design using angular transformations. Statistical analysis and Interpretation of results were done by calculating values of C.D. (critical difference) at 5% level of significance through analysis of variance technique as described by Gomez and Gomez [10]

3. Results and Discussion

3.1 Effect of cow dung smoke on the mortality of different storage insect pests

The data recorded on the mortality of insect pests of stored wheat under smoke and without smoke treatments is presented in Table-1 which showed no insect mortality after one hour of smoke treatments. A negligible insect mortality of *S. oryzae* (1.33%) and (2.00%) under cow dung and neem leaves smoke treatments and (2.66%) mortality of 3rd instar of *C. cephalonica* was observed under neem leaves smoke treatment. After 12h of smoke exposure, 13.33% mortality of both *S. oryzae* and *R. dominica* was observed under neem leave smoke treatments. On the other hand, no insect mortality was observed without smoke under air tight conditions after 1h, 6h and 12h. The insect mortality was increased to 22.67%, 25.33% and 22.00% in neem leaves smoke treated *S. oryzae, R. dominica* and 3rd instar larvae of *C. cephalonica*, respectively after 24h of smoke exposure. A drastic increase in insect mortality was observed after 48h of neem leave smoke exposure with highest per cent mortality in adult moths of *C. cephalonica* (52.00) followed by *R. dominica* (44.00) on the other hand cow dung smoke caused 38.67% and 36.00% mortality in adult moths of *C. cephalonica* and *S. oryzae* in comparison to very less mortality (2.00% to 9.33%) of smoke treated insects was recorded in in without smoke treated wheat seeds. Whereas, under non- smoke conditions, the mortality of insects was observed significantly very less, only after 48h of airtight conditions, which was recorded (9.33%) in adult weevils of *S. oryzae* followed by adult moths of *C. cephalonica* (7.33%), adult beetles of *R. dominica* (5.33%), with the least mortality was recorded in 5th instar larvae of *C. cephalonica* (2.00%).

After 72h of smoke exposure, the significantly highest mortality was noted in neem leaves and cow dung smoke treated adult moths of *C. cephalonica* (68.00%) and (60.00%), respectively followed by mortality in neem leaves smoke treated *S. oryzae* (52.67%) followed by *R. dominica* (53.33). The neem smoke was proved lethal to all tested storage insect pests as more than 50.00% insect mortality was observed after 96 h of exposure with the highest mortality (91.33%) of adult moths of *C. cephalonica* followed by adult weevils of *S. oryzae* (76.67%), adult beetles of *T. casteneum* (63.33%), adult beetles of *R. dominica* (58.00%). The cow dung smoke caused the highest mortality (82.67%) of adult moths of *C. cephalonica* followed by *S. oryzae* (66.00%), *T. casteneum* (58.00%), 3rd instar larvae of *C. cephalonica* (52.67%) with the least mortality was recorded in tested insects, *R. dominica* (49.33%) and 5th instar of *C. cephalonica* (46.00%). While, significantly less insect mortality was recorded without smoke in adult beetles of *T. casteneum* (10.00) followed by beetle of *R. dominica* (14.66%) with the highest mortality in adult moths of *C. cephalonica* (27.33%).

After 120 h of exposure, in comparison to smoke treated insects, the mortality of the all of the types of storage insect kept under airtight conditions without smoke was found significantly less as recorded in adult beetles of *T. casteneum* (18.00%) followed by *R. dominica* (20.00%), 5th instar larvae of *C. cephalonica* (25.33%), 3rd instar of *C. cephalonica*

(30.67%), *S. oryzae* (32.67%) with highest mortality (47.33%) in adult moths of *C. cephalonica.* Whereas on the other hand, cow dung smoke caused 98.67% mortality of adult moths of *C. cephalonica* followed by *S. oryzae* (78.67%), 3rd instar of *C. cephalonica* (71.33%), *T. casteneum* (67.33%), 5th instar of *C. cepahlonica* (64.00%) with least mortality found in *R. dominica* (62.67%). The neem leaves smoke proved lethal to adult moths of *C. cephalonica* with mortality (99.00%) followed by adult weevils of *S. oryzae* (86.67%), adult beetles of *R. dominica* (74.00%), adult beetles of *T. cateneum* (72.00%),3rd instar larvae of *C. cephalonica* (70.67%), with the least insect mortality was observed in 5th instar larvae of *C. cephalonica* (68.00%).

These findings are in agreement with the other reports, which showed that smoke possessed fumigant toxicity to various insect pests. Among the potential new fumigants for controlling pests in stored products, smoke (CO_2) is a good alternative to methyl bromide and phosphine; the traditional fumigants [11]. The efficacy of smoke generated by burning cow dung cake containing 4% CO_2 gave 31.5, 32.0, 37.8, 57.8 and 80.0 per cent mortality of adult *Callosobruchus maculatus* at 24, 48, 72, 96 and 120 hr. exposure, respectively [12]. The use of carbon dioxide is safe, has no toxicological risks and main causes of its toxicity to insects is the stimulus to the opening of spiracles, which induces water loss and dessication [13]. The complete mortality of eggs of *C. maculatus* was observed by CO_2 treatment under pressure [14] with more than 80%, mortality of pulse beetle, *C. maculatus* at 24 h exposure of cow dung cake smoke [15]. A fumigant toxicity of neem formulations *viz.* ware house neem I (mist and spray) and ware house neem II (thermal fog) both containing Azadirachtin-1500 ppm against *S. oryzae* and *R. dominica* with more than 90% mortality of both insects in stored maize [16]. The adults of *S. oryzae* and *R. dominica* were exposed to smoke generated from partial combustion of paddy husk for 2 1/2-3 hours and found it responsible for mortality of both insects under storage conditions [17]. The effect of smoke generated by burning of different parts (root, stem, leaf and fruit) of plant, *Aerva tomentosa* was evaluated against *R. dominica* which gave 40 – 50% adult mortality in 48 h of treatment [18].

3.2 Effect of Smoke on quality parameters of seeds under different exposure time period

The wheat seeds were treated with cow dung and neem leaves smoke separately for different exposure periods for germination and post germination response. The data given in Table- 2 showed the effect of cow dung and neem leaves smoke on the quality of wheat seeds at different exposure time. Both treated and untreated seeds indicated variability in germination percentage, germination index, seedling vigor index, root and shoot length in response to smoke treatments.

The data clearly revealed that seeds treated with smoke showed the best germination response as compared to untreated seeds where germination response was less. The germination response of smoke treated wheat seeds was found 100% after 1, 6 and 12 h of exposure of cow dung smoke whereas 96% of germination of smoke treated seeds were found after 24and 48 h of exposure with 94% seed germination was obtained after 72, 96 and 120h of exposure of smoke. On the other hand, the neem leaves smoke treated wheat seeds showed 100 germination after 6h of exposure time which was reduced to 98% after 12, 24, 48 h to 94% after 72, 96 and 120h of exposure period with 90% germination was observed in untreated wheat. The germination index of smoke treated wheat seeds was calculated 5.0 after 12h of exposure whereas it was ranged from 4.7 to 4.9 after 48h, 72, 96 and 120h of exposure period with the least germination index 4.5 was recorded in untreated wheat seed. The highest mean root length was measured 6.53cm and 6.35cm after 12h of cow dung and neem leaf smoke exposure, respectively. The highest root length was measured in cow dung smoke treated wheat seeds (6.53cm) followed by neem leaves smoke treated seeds (6.25cm) after 12h of exposure. The root length was found quite more in smoke treated wheat seeds after 96 and 120h of exposure (6.12cm and 5.76cm) whereas among the other treatments it was ranged from 4.92cm to 6.53cm after 1h and 72h of exposure of smoke in comparison to root length in untreated control (4.32cm).

Similarly, the shoot length was measured the highest in cow dung smoke treated wheat seeds (7.78cm) followed by neem leaves smoke treated seeds (7.48cm) after 12h of exposure while the shoot length was again measured more (7.68cm) in the wheat seeds treated with cow dung followed by shoot length (7.44cm) measured in neem smoke treated seeds after 120h of exposure. The overall shoot length under different exposure times was ranged from 6.26 cm to 7.68cm with the least shoot length (6.24cm) in untreated wheat seeds. The vigour index (VI) values which was found the highest (1431) and (1345) after 12hr. exposure of wheat seeds to cow dung and neem smoke, respectively. The vigour index (VI) values which was found the highest (1431) and (1345) after 12hr. exposure of wheat seeds to cow dung and neem smoke, respectively.

The highest vigour index (1431.00) was calculated in cow dung smoke treated wheat seeds followed by neem leaves smoke treated seeds (1345.54) after 12 h of exposure whereas the overall vigour index under different exposure time periods of both cow dung and neem leaves smoke was ranged from 1098.58 to 1270.08 in comparison to the least vigour index obtained (950.40) in untreated wheat seeds which clearly showed the efficacy of smoke in promoting the vigourity of wheat seeds. The significance of viability was calculated the highest (1.11) for both cow dung and neem leaves smoke treated wheat seeds after 1,6 and 12 h. of exposure whereas among the other exposure times it was varied from 1.04-1.09 which showed the positive effects on the germination and post germination response of smoke treated wheat seeds.

The present study made on the effect of smoke on quality of wheat seeds was similar to the studies made by others. In the early 1990‘s, smoke was identified as an important germination tool [19]. In wheat the bioactivity of plant derived smoke was first identified [20] who conducted bioassays on wheat coleoptiles and observed a positive and stimulatorv effect of plant derived smoke on germination percentage, germination index, seedling vigor index and root shoot length of wheat seeds for 1 h exposure period·· Plant derived smoke play a positive role in enhancing seed germination of many hard-to-germinate and rare and threatened species [21]. Maize kernels treated with smoke produced more vigorous seedlings (heavier and taller) [22]. He noticed that concentrated smoke solution have inhibitory effect on fresh weight of root and shoot.

Smoke, however, not only influences germination but importantly it also stimulates seedling vigor (post-germinative growth) [23]. Plant derived smoke stimulate and promote germination response as it increase seed sensitivity to endogenous GA4 [24] and therefore enhance seed germination. A positive effect of smoke solutions derived from different

weeds plants *i.e*. *Asphodelus, Avena, Galium, Parthenium* and *Phalaris* was reported on quality of wheat seeds with significantly increased seed germination at 12, 24 and 36h [25]. The plant derived smoke was found effective in increasing per cent germination and vigour index of non-imbibed wheat seeds [26].

Table 1: Effect of Smoke on mortality of storage insect pests in wheat grains under airtight conditions

S. No.	Insect species	Smoke Treatment	Per cent mortality (hours after treatment)							
			1	6	12	24	48	72	96	120
1.	*Sitophilus oryzae*	Cow dung smoke	0.00	1.33 (5.56)	4.66 (12.42)*	8.00 (16.46)	18.00 (25.11)	34.00 (35.70)	66.00 (54.27)	78.67 (62.46)
		Neem leaves Smoke	0.00	2.00 (8.59)	13.33 (21.39)	22.66 (28.40)	36.00 (36.88)	52.67 (46.25)	76.67 (61.08)	86.67 (68.55)
		Without smoke	0.00	0.00	0.00 (0.00)	4.66 (12.41)	9.33 (17.76)	14.67 (22.48)	26.00 (30.66)	32.67 (34.75)
2.	*Rhyzopertha dominica*	Cow dung smoke	0.00	0.00	6.66 (14.93)	14.00 (22.00)	28.00 (31.96)	40.00 (39.19)	49.33 (44.60)	62.67 (52.31)
		Neem leaves Smoke	0.00	0.00	13.33 (21.41)	25.33 (30.20)	44.00 (41.55)	53.33 (46.74)	58.00 (49.60)	74.00 (59.36)
		Without smoke	0.00	0.00	0.00 (0.00)	0.00 (0.65)	5.33 (13.33)	10.00 (18.45)	14.67 (22.14)	20.00 (26.57)
3	*Tribolium casteneum*	Cow dung smoke	0.00	0.00	2.67 (9.33)	14.67 (22.48)	21.33 (27.50)	36.67 (37.24)	58.00 (49.58)	67.33 (55.12)
		Neem leaves Smoke	0.00	0.00	4.00 (11.55)	16.00 (23.59)	24.00 (29.34)	48.00 (43.82)	63.33 (52.66)	72.00 (58.06)
		Without smoke	0.00	0.00	0.00 (0.00)	0.00 (0.00)	3.33 (10.49)	5.33 (13.33)	10.00 (18.46)	18.00 (25.12)
4	*Corcyra cephalonica* Adult moths	Cow dung smoke	0.00	0.00	6.00 (16.45)	13.33 (23.59)	38.67 (46.15)	60.00 (55.55)	82.67 (72.85)	98.67 (84.23)
		Neem leaves Smoke	0.00	0.00	8.00 (16.45)	16.00 (23.59)	52.00 (46.15)	68.00 (55.55)	91.33 (72.85)	99.00 (84.23)
		Without smoke	0.00	0.00	2.00 (8.59)	4.67 (12.42)	7.33 (15.70)	16.00 (23.58)	27.33 (31.49)	47.33 (43.39)
5	*Corcyra cephalonica* 3rd instar larvae	Cow dung smoke	0.00	0.00	7.33 (15.68)	15.33 (23.04)	24.00 (29.34)	28.66 (32.34)	52.67 (46.51)	71.33 (57.63)
		Neem leaves Smoke	0.00	2.67 (9.33)	9.33 (17.77)	22.00 (27.98)	27.33 (31.50)	36.00 (36.88)	56.66 (48.81)	70.66 (57.17)
		Without smoke	0.00	0.00	0.00 (0.00)	0.67 (4.55)	2.67 (9.33)	6.00 (14.20)	20.00 (26.57)	30.67 (33.43)
6	*Corcyra cephalonica* 5th instar larvae	Cow dung smoke	0.00	0.00	0.00 (0.00)	9.33 (17.76)	12.67 (20.81)	24.00 (29.35)	46.00 (42.71)	64.00 (53.14)
		Neem leaves Smoke	0.00	0.00	8.00 (16.44)	14.67 (22.32)	14.67 (22.48)	26.67 (31.06)	58.00 (49.85)	68.00 (55.56)
		Without smoke	0.00	0.00	0.00 (0.00)	0.00 (0.00)	2.00 (8.15)	5.33 (13.33)	18.00 (25.12)	25.33 (30.20)
	CD (P=0.05)	Cow dung smoke	-	-	0.044 (0.514)	0.049 (0.044)	0.048 (0.032)	0.071 (0.026)	0.117 (0.069)	0.305 (0.052)
		Neem leaves Smoke	-	-	0.030 (0.034)	0.267 (0.217)	0.276 (0.012)	0.345 (0.196)	0.133 (0.071)	0.064 (0.069)
		Without smoke	-	-	-	0.041 (0.610)	0.307 (0.050)	0.036 (0.037)	0.206 (0.206)	0.211 (0.129)

*Figures in the parentheses are angular transformed values

Table 2: Effect of Smoke on germination and post germination response of wheat seeds after different exposure time

S. No.	Smoke exposure in (hour)	Smoke Treatment	Germination (%)	Germination index	Root length (cm)	Shoot length (cm)	Vigour index	Significance of viability
1	1	Cow dung smoke	100.00	5.0	4.92	7.14	1206.00	1.11
		Neem leaves Smoke	100.00	5.0	5.33	6.73	1206.00	1.11
2	6	Cow dung smoke	100.00	5.0	5.34	6.65	1199.00	1.11
		Neem leaves Smoke	100.00	5.0	5.38	6.35	1173.00	1.11
3	12	Cow dung smoke	100.00	5.0	6.53	7.78	1431.00	1.11
		Neem leaves Smoke	98.00	4.9	6.25	7.48	1345.54	1.09
4	24	Cow dung smoke	96.00	4.8	5.28	7.25	1202.88	1.07
		Neem leaves Smoke	98.00	4.9	5.98	6.98	1270.08	1.09
5	48	Cow dung smoke	96.00	4.8	5.57	7.33	1238.40	1.07
		Neem leaves Smoke	98.00	4.9	4.64	6.57	1098.58	1.09
6	72	Cow dung smoke	94.00	4.7	6.24	6.75	1221.06	1.04
		Neem leaves Smoke	94.00	4.7	5.67	6.26	1121.42	1.04
7	96	Cow dung smoke	94.00	4.7	5.64	6.32	1124.24	1.04
		Neem leaves Smoke	94.00	4.7	6.12	6.68	1203.20	1.04
8	120	Cow dung smoke	94.00	4.7	5.76	7.68	1263.36	1.04
		Neem leaves Smoke	94.00	4.7	5.46	7.44	1212.60	1.04
9	-	Without smoke	90.00	4.5	4.32	6.24	950.40	-

4. Conclusion

The present investigation clearly revealed the efficacy of cow dung and neem leaves smoke against insect pests of stored wheat *viz. S. oryzae*, *R. dominica*, *C. cephalonica* and *T. casteneum* as more than 50% insect mortality was observed after 72h to 96h of exposure of smoke. On the other hand, smoke was also found very effective for enhancing the germination of treated wheat seeds with more root and shoot length which ultimately gave high vigour index and significance of viability under airtight conditions. The present hazardous fumigants for eco-friendly management of insect pests of stored wheat without impairing the quality of wheat seeds. However, it is concluded from the present study, that smoke generated by burning of cow dung cake and neem leaves was found lethal to storage insect pests but has stimulatory effect on germination and post germination response of seeds and as being cost effective, easily available, eco-friendly, non-hazardous and easy to adopt by small-scale farmers, can therefore be used as an alternatives to synthetic insecticides and fumigants for safe store of wheat grains under storage conditions.

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